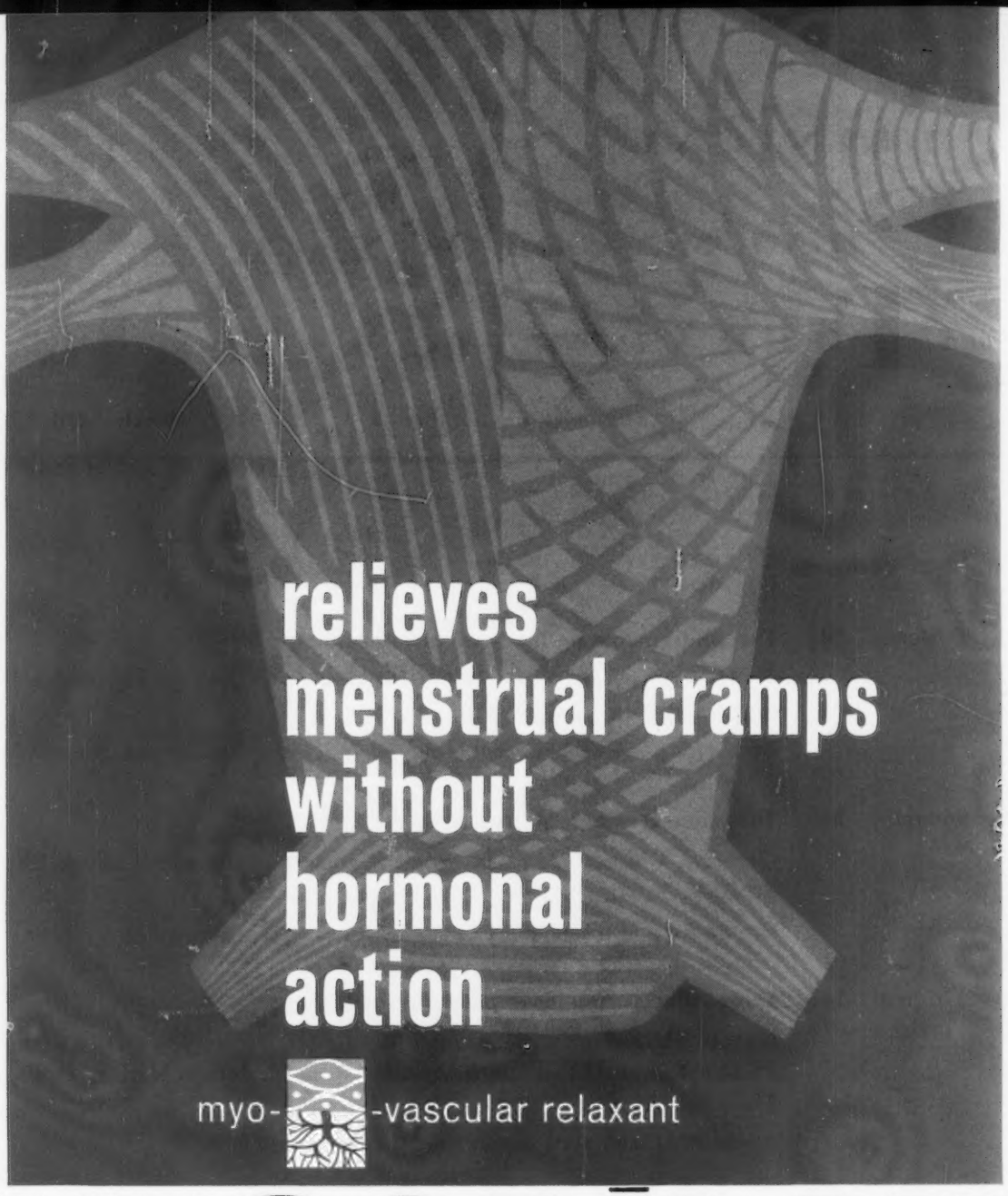
CLINICAL PHARMACOLOGY and THERAPEUTICS

*volume* 2 *number* 2 **March-April 1961**

## Table of contents



**continued on page 3**




# VASODĪLAN®

Isoxsuprine hydrochloride, Mead Johnson

**relieves uterine spasm and hypermotility by *direct* relaxant action on uterine muscle***
**proved effective clinically with a low incidence of side effects***

**dosage:** For menstrual cramps, give 10 or 20 mg. (1 or 2 tablets) three or four times daily 24 to 72 hours prior to expected onset of menstruation. Continue until pain has been averted.

**side effects:** Few side effects occur when given in recommended oral doses. Occasional palpitation and dizziness can usually be controlled by dosage adjustment. Single intramuscular doses of 10 mg. or more may result in hypotension or tachycardia.

**contraindications:** There are no known contraindications to oral administration of VASODĪLAN in recommended doses.

**cautions:** VASODĪLAN should not be given immediately postpartum or in the presence of arterial bleeding. Parenteral administration is not recommended in the presence of hypotension or tachycardia. Intravenous administration is *not* recommended because of the increased likelihood of side effects.

**supplied:** 10 mg. tablets, bottles of 100; 2 cc. ampuls (5 mg./cc.) for *intramuscular* use, boxes of 6.

*Voulgaris, D. M.: Obst. & Gynec. *15*:220-222 (Feb.) 1960.

115R61

Mead Johnson
Laboratories

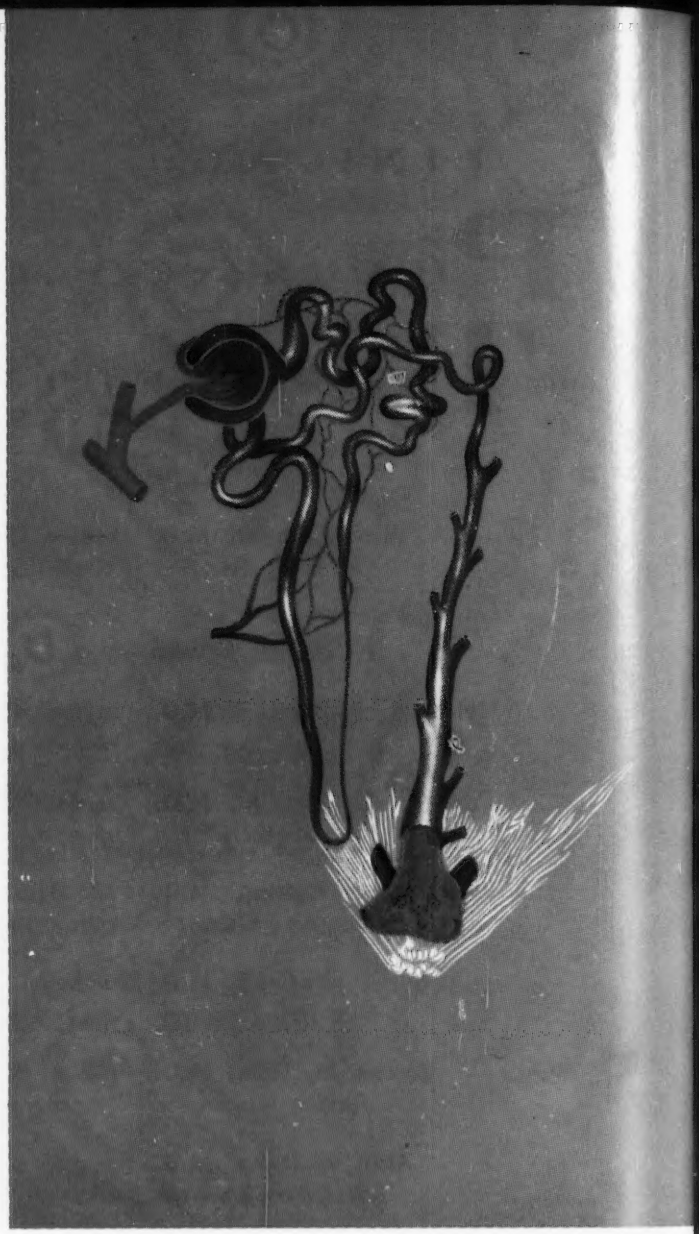
*Symbol of service in medicine*

*Volume 2, Number 2, March-April,* 1961, CLINICAL PHARMACOLOGY AND THERAPEUTICS. *Published bimonthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo. Second class postage paid at St. Louis, Mo. Subscription rates: United States, its possessions, and Canada* $12.50; *Latin America and Spain* $13.50; *other countries* $14.00. *Students, interns, and resident physicians: United States, its possessions, and Canada* $7.50; *Latin America and Spain* $8.50; *other countries* $9.00. *Single copies* $2.50 *postpaid.*

# CLINICAL PHARMACOLOGY and THERAPEUTICS



*Continued on page 7*



## *on the pathogenesis of pyelonephritis:*

"An inflammatory reaction here [renal papillae] may produce sudden rapid impairment of renal function. One duct of Bellini probably drains more than 5000 nephrons. It is easy to see why a small abscess or edema in this area may occlude a portion of the papilla or the collecting ducts and may produce a functional impairment far in excess of that encountered in much larger lesions in the cortex."[1]

The "exquisite sensitivity"[2] of the medulla to infection (as compared with the cortex), highlights the importance of obstruction to the urine flow in the pathogenesis of pyelonephritis. "There is good cause to support the belief that many, perhaps most, cases of human pyelonephritis are the result of infection which reaches the kidney from the lower urinary tract."[3]

## *to eradicate the pathogens no matter the pathway*

# FURADANTIN®

brand of nitrofurantoin

High urinary concentration • Glomerular filtration plus tubular excretion • Rapid antibacterial action • Broad bactericidal spectrum • Free from resistance problems • Well tolerated—even after prolonged use • No cross resistance or cross sensitization with other drugs

*Average Furadantin Adult Dosage:* 100 mg. tablet q.i.d. with meals and with food or milk on retiring. *Supplied:* Tablets, 50 and 100 mg.; Oral Suspension, 25 mg. per 5 cc. tsp.

References: 1. Schreiner, G. E.: A.M.A. Arch. Int. M. **102**:32, 1958. 2. Freedman, L. R., and Beeson, P. B.: Yale J. Biol. & Med. **30**:406, 1958. 3. Rocha, H., et al.: Yale J. Biol. & Med. **30**:341, 1958.

NITROFURANS—a *unique* class of antimicrobials

EATON LABORATORIES, DIVISION OF THE NORWICH PHARMACAL COMPANY, NORWICH, N. Y.

# CLINICAL PHARMACOLOGY and THERAPEUTICS

*Editor*

**Walter Modell, M.D.**
*1300 York Avenue*
*New York 21, New York*

*Assistant Editor*

**Edel Berman, M.B., Ch.B.**
*1300 York Avenue*
*New York 21, New York*

*Publisher*

**The C. V. Mosby Company**
*3207 Washington Boulevard*
*St. Louis 3, Missouri*


## *Editorial Communications*

*Clinical Pharmacology and Therapeutics* is a bimonthly journal devoted to the publication of original articles and reviews dealing with the effects of drugs in man. Articles are accepted on the condition that they are contributed solely to *Clinical Pharmacology and Therapeutics*. Neither the editors nor the publisher accept responsibility for the views and statements of authors expressed in their communications. Manuscripts submitted for publication should be sent to the Editor.

**Manuscripts.** Manuscripts should be typewritten on one side of the paper only, with double spacing and liberal margins. Manuscripts should be submitted in duplicate, i.e., an original and one carbon copy. Failure to submit a duplicate copy prolongs editorial decisions considerably.

**Summaries.** A summary of not more than 250 words must also be submitted on a separate sheet in duplicate. It will appear under the title of the published article.

**References.** References should be placed at the end of the article (not as footnotes on each page), listed alphabetically and numbered accordingly. They should conform to the style of the Quarterly Cumulative Index Medicus and include complete pagination. Personal communications should be excluded from the references and used, if at all, only as footnotes. In press references may be used only if the accepting journal is indicated.

**Illustrations.** Illustrations accompanying manuscripts should be numbered and marked lightly on the back with the author's name. Legends should be typed on a separate sheet. Authors should indicate on the manuscript the appropriate positions of tables and text figures.
A reasonable number of halftone illustrations will be reproduced free of cost to the author, but special arrangements must be made with the Editor for color plates, elaborate tables, or extra illustrations. Copy for zinc cuts (such as pen drawings and charts) must be drawn in India ink and lettered in India ink or black typewriter ribbon. Only good photographic prints and original drawings should be supplied for halftone work.

**Correspondence.** Concise informative correspondence may be sent to the Editor for publication. Interesting letters, or portions thereof, will be published, but unpublished letters will not be returned.

**Exchanges.** Contributions, letters, exchanges, reprints, and all other communications should be sent to the Editor.

**Review of Books.** Books and monographs, native and foreign, will be reviewed according to their merits and as space permits. Books may be sent to Dr. Walter Modell, 1300 York Avenue, New York 21, New York.

**American Therapeutic Society.** Official notices of the Society will appear in the Journal.

**Drug Nomenclature.** Only generic and chemical names of drugs are permitted. A proprietary equivalent may be indicated *once*, in a footnote.


Published bimonthly. Subscriptions may begin at any time

*Subscription rates*

United States, its possessions, and Canada *$12.50*
Latin America and Spain *$13.50*
Other countries *$14.00*

*Students, interns, and resident physicians*

United States, its possessions, and Canada *$7.50*
Latin America and Spain *$8.50*
Other countries *$9.00*

*Single copies*, postpaid *$2.50*

Remittances for subscriptions should be made by check, draft, post office or express money order, payable to this Journal


## *Business Communications*

All communications in regard to advertising, subscriptions, change of address, etc., should be addressed to the Publishers.

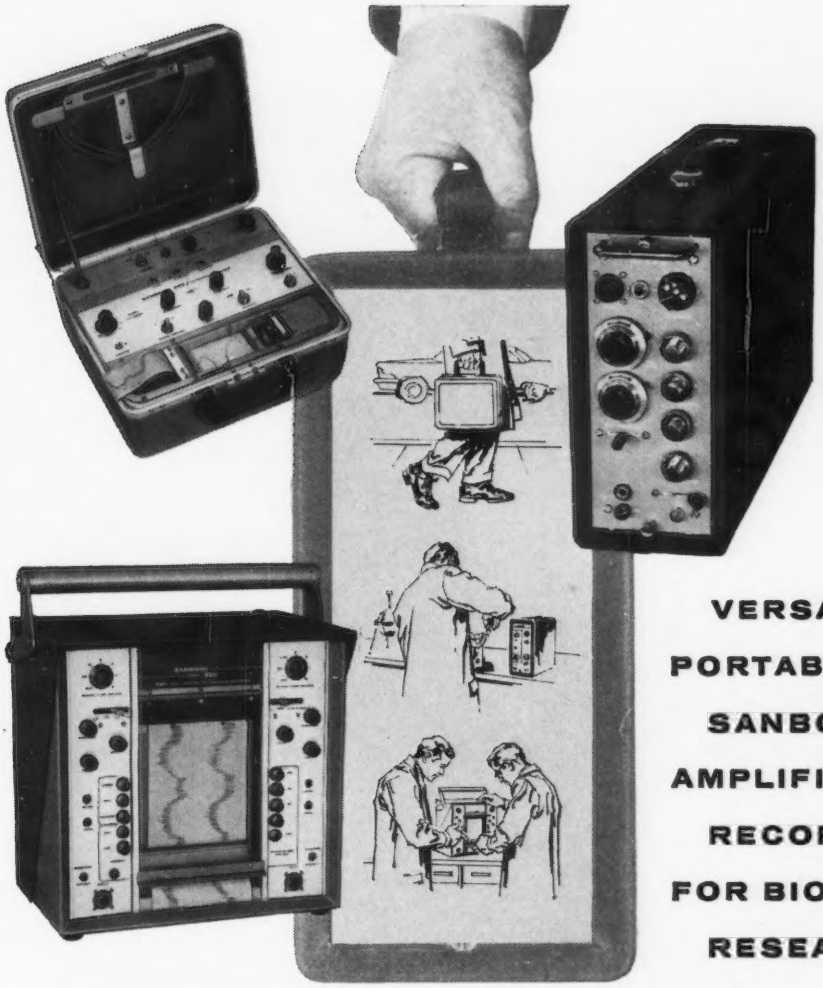
**Reprints.** Reprints of articles must be ordered directly through the publishers, The C. V. Mosby Company, 3207 Washington Boulevard, St. Louis 3, Missouri, who will send their schedule of prices. Individual reprints of an article must be obtained through the author.

**Publication Order.** This Journal is published bimonthly, January through November. The volume index is in the November issue.

**Change of Address Notice.** Six weeks' notice is required to effect a change of address. Kindly give the exact name under which a subscription is entered, and the full form of both old and new addresses, including the post office zone number.

**Advertisements.** Only articles of known scientific value will be given space. Forms close first of month preceding date of issue. Advertising rates and page sizes on application.

**Bound Volumes.** Publishers' Authorized Bindery Service, 430 West Erie Street, Chicago 10, Illinois, will be glad to quote prices for binding volumes in buckram.



## VERSATILE, PORTABLE SANBORN AMPLIFIER RECORDERS FOR BIOPHYSICAL RESEARCH

Any one of these portable amplifier recorders will provide your laboratory with greater recording flexibility and operating economy. Select either a one or two channel model with electronics for either DC or carrier amplification, for such applications as routine recording from transducers . . . monitoring output of laboratory instruments such as Sanborn "450" portable amplifiers (self-contained units with built-in power supplies, available in DC Coupling, Carrier, ECG/General Purpose, Low Level and other types), vital capacity spirometers, pH meters, etc. . . . or for direct-writing recording of one or two channels of a photographic recording system (e.g., Sanborn 564 and 558M) or a visual monitoring system (e.g., Sanborn 760 and 762).

FOR GENERAL PURPOSE DC RECORDING

*The Model 299—21 lb., brief case, 1-channel recorder* . . . 5 and 50 mm/sec or 2.5 and 25 mm/sec chart speeds . . . sensitivities from 10 volts to 10 mv/div . . . frequency response 0 to 100 cps, down 3 db at 10 div peak-to-peak . . . inkless recording.

*Model 320—2 complete channels in a one-cubic-foot package* — separate, current-feedback amplifiers with floating and guarded inputs . . . sensitivities from 2 volts to 0.5 mv/mm . . . full 50 mm for each channel . . . frequency response 0-125 cps, down 3 db at 10 mm peak-to-peak . . . choice of speed ranges—50, 25, 5, 2.5 mm/sec or 100, 20, 5, 1 mm/sec . . . inkless records. Model 322 also available, for low gain 2-channel recording.

FOR AC CARRIER AMPLIFIER RECORDING

*Model 301* — single-channel portable — same construction, physical specifications and chart speed options as Model 299. Self-contained AC carrier amplifier recorder . . . sensitivities to 10 microvolts rms/div . . . frequency response 0-100 cps, down 3 db, 10 div peak-to-peak . . . internal 2400 cps carrier frequency . . . uncalibrated zero suppression. For similar applications but in *two channels*, Model 321 is available as a carrier amplifier version of the Model 320 system.

Contact your nearest Sanborn Branch Office or Service Agency for complete details, or write Research Instrument Sales Manager:

MEDICAL DIVISION

**SANBORN COMPANY**

175 Wyman St., Waltham 54, Massachusetts

## Erythropoietin—a significant discovery in clinical hematology

A wealth of evidence now confirms the fact that red blood cell production is controlled by the hormone erythropoietin.[1-3] Demonstrated in human plasma,[4] erythropoietin has been shown to produce reticulocytosis,[1,5-7] increase utilization of the $Fe^{59}$ isotope, and increase erythrocyte precursors in marrow cultures.[3,8]

# ERYTHROPOIETIN FOUND TO CONTROL RED CELL FORMATION

**erythropoietin levels—new criteria in diagnosis of anemia** — Increased erythropoietin blood levels can be demonstrated in severe anemia and following the start of accelerated formation.[9] Soon thereafter, the effect of the higher levels appears as an increased erythroid marrow activity.[10] Since the hemopoietic marrow is capable of producing more red cells than normally required, many anemias may be due to inadequate erythropoietin levels—a result of subnormal production or excessive excretion.

**how does erythropoietin affect iron metabolism?** Absorption and utilization of iron are dependent upon the rate of bone marrow erythropoiesis which, in turn, is dependent upon erythropoietin levels.[11,12] Thus, the demand for iron created by accelerated erythropoiesis is satisfied by both increased gastrointestinal absorption and mobilization of storage iron. Inadequate erythropoietin levels would seemingly account for the frequently disappointing results with the use of iron alone in many of the anemias.

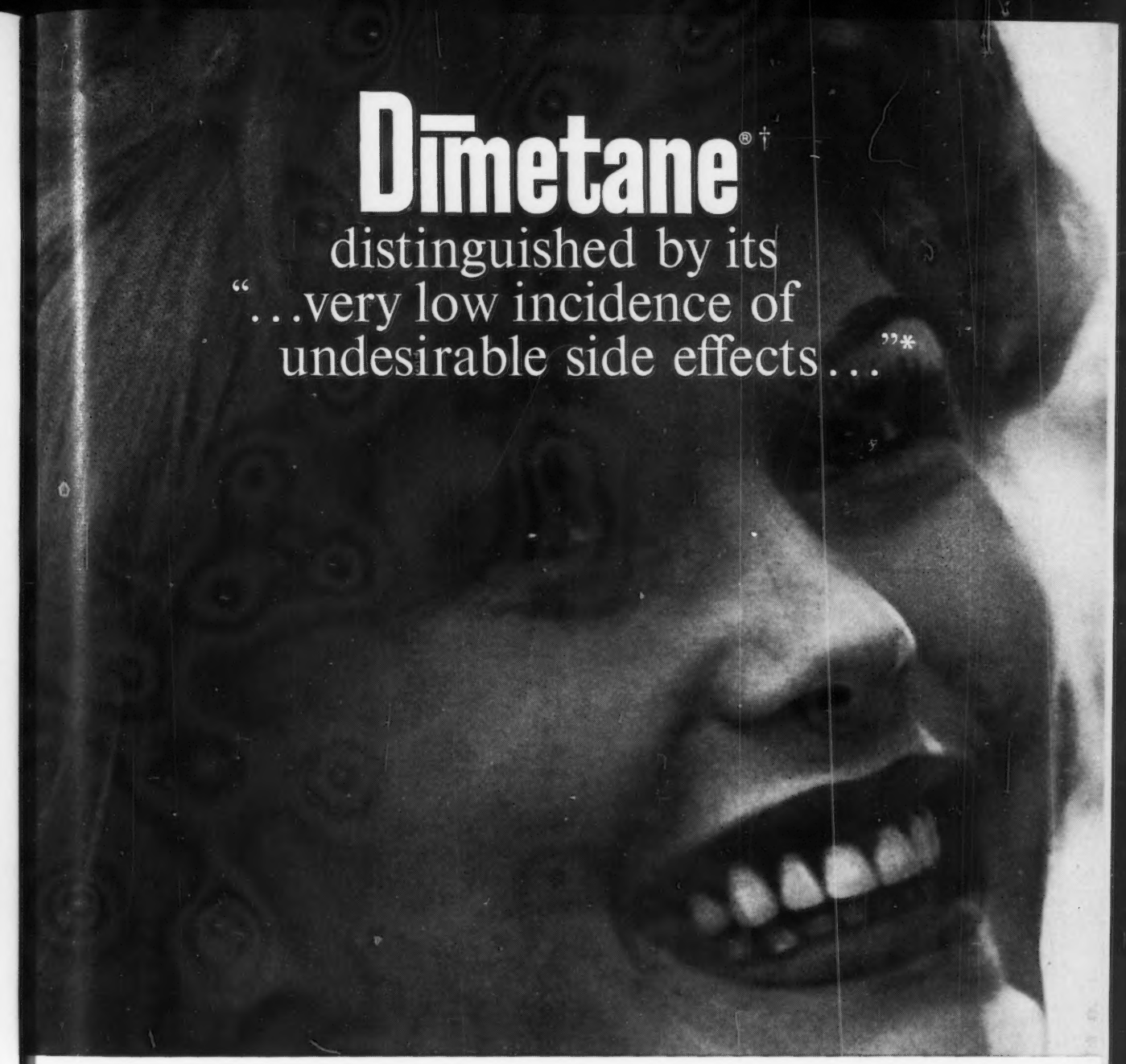
**can medication increase erythropoietin levels?** Cobalt has been shown to be strikingly effective in increasing the production of erythropoietin.[13,14] Cobalt-enhanced erythropoietin accelerates red cell production and improves iron utilization with a subsequent increase in hemoglobin and erythrocytes. The new concepts of the cause, diagnosis, and management of anemia may now be applied clinically on the sound basis of extensive studies published on RONCOVITE® —MF,* the therapeutic cobalt-iron hematinic.

(1) Gordon, A. S.: Physiol. Rev. **39**:1, 1959. (2) Erslev, A. J.: J. Lab. & Clin. Med. **50**:543, 1957. (3) Rosse, W. F., and Gurney, C. W.: J. Lab. & Clin. Med. **53**:446, 1959. (4) Gurney, C. W.; Goldwasser, E., and Pan, C.: J. Lab. & Clin. Med. **50**:534, 1957. (5) Rambach, W. A.; Alt, H. F., and Cooper, J. A. D.: Blood **12**:1101, 1957. (6) Gordon, A. S., **et al.**: Proc. Soc. Exp. Biol. & Med. **92**:598, 1956. (7) Erslev, A. J.: Blood **10**:954, 1955. (8) Goldwasser, E.; Jacobson, L. O.; Fried, W., and Plzak, L. F.: Blood **13**:55, 1958. (9) Stohlman, F., Jr., and Brecher, G.: Proc. Soc. Exp. Biol. & Med. **100**:40, 1959. (10) Kraus, L. M., and Kraus, A. P.: Fed. Proc. **18**:1051, 1959. (11) Bothwell, T. H.; Pirzio-Biroli, G., and Finch, C. A.: J. Lab. & Clin. Med. **51**:24, 1958. (12) Beutler, E., and Buttenwieser, E.: J. Lab. & Clin. Med. **55**:274, 1960. (13) Goldwasser, E.; Jacobson, L. O.; Fried, W., and Plzak, L.: Science **125**:1085, 1957. (14) Murdock, H. R., Jr., and Klotz, L. J.: J. Am. Pharm. A. (Scient. Ed.) **48**:143, 1959.

*Cobalt chloride (cobalt as Co 3.7 mg.), 15 mg. ferrous sulfate exsiccated, 100 mg.

V-00361-R 104-R

LLOYD BROTHERS, INC.
Cincinnati, Ohio



# Dimetane®†

## distinguished by its "...very low incidence of undesirable side effects..."*

## even in allergic infants

FROM A CLINICAL STUDY* IN ANNALS OF ALLERGY

| | |
|---|---|
| Patients | 200 infants and children, ages 2 months to 14 years |
| Diagnosis | Perennial allergic rhinitis |
| Therapy | Dimetane Elixir |
| Results | in 149, *good* results / in 40, *fair* results |
| Side Effects | Encountered in *only* 7 patients (in all except one, the side effect was mild drowsiness) |

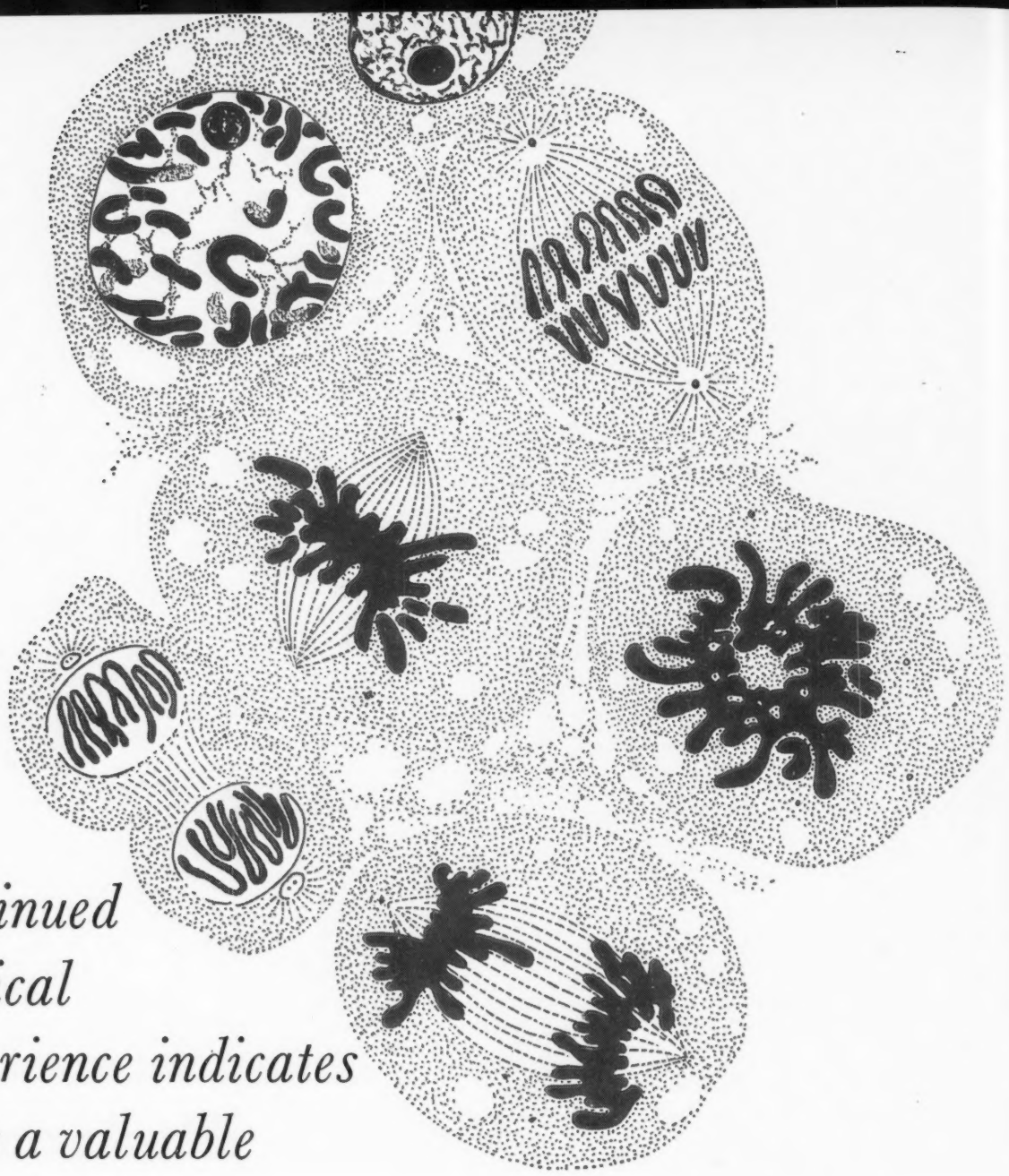
In allergic patients *of all ages,* Dimetane has been shown to work with an effectiveness rate of about 90% and to produce an exceptionally low incidence of side effects. Complete clinical data are available on request to the Medical Department. *Supplied:* DIMETANE Extentabs® (12 mg.), Tablets (4 mg.), Elixir (2 mg./5 cc.), new DIMETANE-TEN Injectable (10 mg./cc.) or new DIMETANE-100 Injectable (100 mg./cc.).

Robins

*MC GOVERN, J. P., MC ELHENNEY, T. R., HALL, T. R., AND BURDON, K.D.: ANNALS OF ALLERGY 17:915, 1959.

**A. H. ROBINS CO., INC., RICHMOND 20, VIRGINIA/ETHICAL PHARMACEUTICALS OF MERIT SINCE 1878**

† PARABROMDYLAMINE MALEATE



*continued clinical experience indicates it as a valuable cytotoxic agent*

# CYTOXAN®

*Cyclophosphamide, Mead Johnson*

FOR PALLIATIVE CHEMOTHERAPY OF CERTAIN TYPES OF MALIGNANT NEOPLASMS

Report from a recent comparative clinical evaluation of two alkylating agents: "With the use of cyclophosphamide [Cytoxan], there is a relative lack of thrombocytopenia and a diminution in gastrointestinal side-effects, so that it may offer therapeutic advantages over other alkylating agents."*

Other Advantages in Clinical Practice: Broad spectrum of application • High therapeutic index • No vesicant activity—may be given orally or parenterally.

*Papac, R.; Petrakis, N. L.; Amini, F., and Wood, D. A.: J.A.M.A. *172*:1387-1391 (March 26) 1960.

**DOSAGE:** *For neoplasms relatively susceptible to Cytoxan* — Patients with lymphomas and other neoplasms believed to be relatively susceptible to Cytoxan therapy are given an initial dose of 2-3 mg./Kg./day intravenously. White blood counts and platelet determinations should be made daily or twice weekly and the dosage adjusted accordingly. Intravenous infusions should be continued for at least 6 days unless otherwise indicated. A leukopenia of between 1500 and 5000 cells per cu. mm. (or lower) may be expected between the tenth and fourteenth day. In the presence of a leukopenia of less than 2000/cu. mm. Cytoxan should be discontinued until the white cell count returns to 2000 to 5000 (usually within a week). Dosage is subsequently adjusted as indicated by the patient's objective response and the leukocyte count. If the patient is subjectively improved, if the size of the tumor has decreased, or if the white cells are satisfactorily maintained between 2000 and 5000/cu. mm. oral dosage may be instituted equivalent to intravenous dosage.

Thrombocytopenia is rarely observed on this regimen. If platelet counts of less than 100,000/cu. mm. are observed, the patient should be watched carefully. If platelets continue to decrease, Cytoxan should be discontinued.

The patient who has had previous treatment with alkylating agents, or x-ray, or is debilitated may be more susceptible to bone marrow depression, and initial Cytoxan doses should be more conservative than the above. Such patients should have more frequent hematologic evaluation. Good medical practice demands access to a reliable hematologic laboratory when using Cytoxan.

*For neoplasms relatively resistant to Cytoxan* — Patients with carcinomas and other malignant neoplasms believed to be less susceptible to Cytoxan therapy are given a dose of 4 to 8 mg./Kg./day intravenously. Unless there are indications to the contrary, this dose is continued for 6 days, then stopped. Leukopenia usually ensues on the tenth to fourteenth day after the first dose of Cytoxan. Thrombocyte reduction is not common, and platelets may actually increase. The leukocyte count promptly returns toward normal levels in most cases, and as it begins to increase, sufficient Cytoxan is administered to maintain it near 2000 to 5000/cu. mm. This may be accomplished by two intravenous injections weekly, or by oral administration, or by a combination of both routes. An oral dosage of 50 to 200 mg. daily or an intravenous injection of 5 mg./Kg. twice weekly will usually suffice.

The platelet and leukocyte counts should be followed carefully, and the prior treatment history of patients carefully evaluated as delineated above.

*Leukopenia as a guide to adequacy of dosage* — The best objective measure for dosage seems to be the number of circulating white blood cells. This is used as an index of the activity of the hematopoietic system, especially the bone marrow. The mechanism by which Cytoxan causes a reduction in the level of white blood cells is not known, but cessation of dosage results in an increase in the level, indicating that the hematopoietic system had not been permanently affected. When large doses (8 mg./Kg./day for 6 days) are given initially, the white cell count falls rapidly. Following the cessation of the 6-day course, the white cells may continue to decline for as long as 8 days and then increase. The reduction of the white cell count during Cytoxan therapy and its subsequent increase when therapy is discontinued can be repeated in the same patient. Maximal reduction in leukocyte count indicates the maximal permissible Cytoxan level for therapeutic effect. Leukopenic patients must be watched carefully for evidence of infection.

Total white blood cell and thrombocyte counts should be obtained 2 or more times weekly in order to evaluate therapy and to adjust dosage.

**SIDE EFFECTS:** Although Cytoxan is related to nitrogen mustard, it has no vesicant effect on tissue. It does not traumatize the vein when injected intravenously, nor does it cause any localized tissue reaction following extravasation. It may be administered intravenously, intramuscularly, intraperitoneally, intrapleurally or directly into the tumor, when indicated. It is apparently active by each of these routes.

Nausea and vomiting are common and depend on dose and on individual susceptibility. However, many investigators accept the nausea and vomiting in favor of maintaining maximal therapy. The vomiting can be controlled with antiemetic agents.

Alopecia is a frequent side reaction to Cytoxan therapy. It has been observed in 28% of the patients studied in this country. The incidence is greater with larger doses. The loss of hair may first be noted about the 21st day of therapy and may proceed to alopecia totalis. This effect is reversed following discontinuance of Cytoxan; during reduced maintenance therapy, hair may reappear. It is essential to advise the patient in advance concerning this effect of the drug.

Dizziness of short duration and of minor degree has occasionally been reported.

Leukopenia is an expected effect and can be used as a guide to therapy. Thrombocytopenia may occur, especially after large doses. The leukocyte or platelet counts of an occasional patient may fall precipitously after even small doses of Cytoxan, as with all alkylating agents. The drug should be discontinued in such patients and reinstituted later at lower dosage after satisfactory hematologic recovery has occurred. Prior treatment with x-ray or with other chemotherapeutic agents frequently causes an earlier or exaggerated leukopenia or thrombocytopenia after Cytoxan medication. Only rarely has there been a report of erythrocyte or hemoglobin reduction.

**ADMINISTRATION:** Add 5 cc. sterile water (Water for Injection, U.S.P.) to 100 mg. of Cytoxan in the sterile vial (add 10 cc to 200 mg. vial). Shake, allow to stand until clear, remove with sterile syringe and needle and inject.

The freshly prepared solution of Cytoxan may be administered intravenously, intramuscularly, intraperitoneally, intrapleurally, or directly into the tumor. The solution should be administered promptly after being made but is satisfactory for use for three hours after preparation.

If the patient is receiving a parenteral infusion, the Cytoxan solution may be injected into the rubber tubing if the solution is glucose or saline.

No thrombosis or thrombophlebitis has been reported from injections of Cytoxan. Extravasation of the drug into the subcutaneous tissues does not result in local reactions.

**PRECAUTIONS:** Cytoxan should not be given to any person with a severe leukopenia, thrombocytopenia, or bone marrow infiltrated with malignant cells. It may be given with suitable precautions to patients who have had recent x-ray treatment, recent treatment with a cytotoxic agent, a surgical procedure within 2-3 weeks, or debilitated patients.

**AVAILABILITY:** Cytoxan is available as follows:

Cytoxan for Injection, 100 mg., a sterile dry-filled vial containing 100 mg. cyclophosphamide and 45 mg. sodium chloride. Packaged, 12 vials per carton.

Cytoxan for Injection, 200 mg., a sterile dry-filled vial containing 200 mg. cyclophosphamide and 90 mg. sodium chloride. Packaged, 12 vials per carton.

Cytoxan Tablets for oral administration, 50 mg., white, round tablets, flecked with blue for easy identification. Packaged, 100 tablets per bottle.

For a copy of the Cytoxan brochure, or other additional information on Cytoxan, communicate directly with the Medical Department (Section A), Mead Johnson Laboratories, Evansville 21, Indiana.

Mead Johnson Laboratories

*Symbol of service in medicine*

17161

If you want an antimicrobial:

1. With outstanding effectiveness
2. With a remarkable safety record
3. That need be given only once a day
4. Which is "kind to the purse"

change to

# MADRIBON

MADRIBON®—2,3-dimethoxy-6-sulfanilamido-1,3-diazine

Now—especially for children

**NEW MADRIBON® CHEWABLE TABLETS**

**Good tasting**—orange flavor
**Easily chewed and swallowed**
**Convenient**—no spillage or waste
**Accurate dosage**—0.25 Gm—scored

Consult literature and dosage information, available on request, before prescribing.

ROCHE
LABORATORIES
Division of Hoffmann-La Roche Inc.

CLINICAL PHARMACOLOGY and THERAPEUTICS

*volume 2* *number 2* **March-April 1961**

## Editorial

### The pharmacologic basis of anesthesiology

An editorial on the pharmacologic basis of anesthesiology should sift the evidence bearing upon the fundamental nature of the anesthetic process. The medical student or the young graduate student in pharmacology will recognize this statement as recasting in modified verbal dress the old, and to some the tedious, subject of "theories of narcosis." Teachers of anesthesiology and pharmacology have been content to examine these theories, old and new, with their students in the hope that they were contributing to the education of the student. In fact, most students appear to gain little from such discussions beyond a summary of the historical background of research into mechanisms. The value of this knowledge will not be denied, but its importance to progress has been overemphasized.

Progress toward understanding the mechanism of the anesthetic process has been slow, to put it generously. Some conceptual advances about general anesthesia, based upon limited data, exist beyond the statement made by Goodman and Gilman in 1955 that "No ready explanation is available . . . to elucidate the manner in which central nervous system depressants produce unconsciousness."[1] It is becoming clearer that the electrical and the chemical activity of the central nervous system respond in a precise and predictable way to anesthetic agents, possibly explaining or at least describing something of their mode of action. For example, Mary Brazier[2] has pointed out in brilliant studies that barbiturate anesthetics cause the block of some pathways, the release from inhibition of others, and the more rigid synchronization of the responses in certain specific pathways. Verzeano[3] showed that this change from wakefulness to sleep (including barbiturate sleep) and from electrical "desynchronization" to "synchronization" is related to the appearance of waves of activity propagated sequentially from neuron to neuron in the diffuse thalamic projection system. These data are cited as illustrations of the new and important quantitative descriptions of the anesthetic process provided by electrophysiologic research. These studies do not, however, provide data on the cellular mechanism of anesthesia.

One might look hopefully to the vigorous research in the field of chemical transmitters which tries to improve understanding of the mechanics of brain and nervous sys-

tem function. The actions of various chemical substances in the brain had been suggested by Paton.[4] Although much has been learned, the precise understanding of normal chemical transmission in the brain awaits more data and more complete interpretation. Understanding chemical transmission is vital to the understanding of the mechanism of anesthesia. Paton summarizes this concept most effectively when he says, "I suppose, putting it bluntly, our attempts to understand anesthetics without knowing the central transmitters are like trying to interpret curare or eserine before the role of acetyl choline was known. It brings to mind a stage set, a spotlight ready, and an audience keenly expectant."[4]

Environmental factors are well known but not considered sufficiently when the pharmacologic basis of anesthesiology is contemplated. Certain aspects of the question resemble feedback systems in the newer conceptual formulations or "vicious" (perhaps beneficial in some instances) cycles in the more orthodox pharmacologic views.

Anesthetics, with rare exceptions, influence respiration and the circulation of the blood. These physiologic systems are critical portals of entry and distribution of the anesthetics. The influence of changes in respiration and the circulation resulting from the action of anesthesia is highly significant. Even the degree of access of anesthetic drugs to the brain is dependent upon the complex interrelationships influencing the uptake of drug from alveoli into the blood, the circulatory pressure which propels the blood to the brain and other organs, and the size of the cerebral blood flow. Drug action, in anesthesia, not only begets drug effect, but it preconditions the drug effects which follow in the immediate time ahead.

The cellular environment in the central nervous system, and probably in other organs, influences the anesthetic process. Among the more obvious factors are the availability of oxygen to the cells, the pH of the cell and the fluid surrounding it, the concentration and nature of electrolytes, the youth or senescence of the tissue, and the presence or absence of disease.

One can see, therefore, a fascinating and complex set of circumstances which accounts for and conditions the pharmacologic basis of anesthesiology. A few of these factors have been considered. They are those related to descriptive neurophysiology, chemical transmission in the nervous system, the physicochemical environment of the cells of the nervous system, and the interesting "change upon change" which is the result of the action of anesthetic drugs upon respiration and the circulation.

### *References*

1. Goodman, L. S., and Gilman, A.: The pharmacological basis of therapeutics, ed. 2, New York, 1955, The Macmillan Company, pp. 3-4.
2. Brazier, M. A. B.: Some action of anesthetics on the nervous system, Fed. Proc. **19**:626-628, 1960.
3. Verzeano, M.: Activity of cerebral neurons in the transition from wakefulness to sleep, Science **124**:366-367, 1956.
4. Paton, W. D. M.: Mechanisms of transmission in the central nervous system, Anaesthesia **14**: 3-27, 1959.

*E. M. Papper, M.D.*

## Commentary

### The measurement of uncertainty

**Gerhard Werner, M.D.** *New York, N. Y.*
*Cornell University Medical College*

To express results of experimentation in statistical notation has become a common practice and standard requirement. Indeed, statistics has been adopted as routine aspect of the conduct of experiments to an extent which makes statistical terminology seemingly self-evident. This clearly indicates how deeply the vocabulary of probabilistic thinking has molded our scientific and "common sense" habits of thought. Yet, usage of statistical terms is not commonly paralleled by an adequate appreciation of magnitude and scope of the intellectual revolution from which statistical methodology arose.

In the beginning of the eighteenth century, the foundation was laid for probabilistic reasoning, then radically novel but now far reaching in its consequences. It must be conceded that the key term of this frame of reasoning can be traced far back in the intellectual history of the West: Aristotle and Cicero are known to have used the idea of probability in an intuitive and not specifically defined form. The attempt to explore the logical depth and pragmatic scope of the idea of probability constitutes an important feature of an emerging philosophy of science which equally breaks with the scientific tradition of the Greeks and with the physics of Laplace, which have in common absolute certainty and predictability as earmarks of truly scientific knowledge. In retrospect, it seems unfortunate that the idea of probability was, and continues to be, employed in many different contexts at first sight not related to each other in any clearly recognizable manner. In large measure, existing confusions and misconceptions can be traced to this circumstance, and the labor of many a philosopher of science was required to contribute revealing a common pattern where multiplicity appears to reign.

Disregarding their occurrence in everyday discourse, statements involving the idea of probability are made in a variety of contexts: these range from a branch of mathematics known as the calculus of probability to the domain of measurements and to structure and content of physical and biologic theories; they include, furthermore, comparisons of rival theories with respect to their likelihood to be correct and thereby extend into the realm of inductive reasoning. Accordingly, probability may designate in quantitative terms strength or degree of a belief (de Morgan), an intuitive relation between propositions (Keynes), or a relative frequency of oc-

Received for publication June 15, 1960.

currence of a certain "event" in an indefinite, hypothetic, class of events (von Mises, Reichenbach).

The question, then, arises: Is there a common denominator and, if so, how do these different ways of forming statements about probabilities relate to each other. To explore this complex of interrelated questions, I propose the following considerations.

There does not appear any cogent reason for viewing the calculus of probability as differing, in design and scope, from any other systematized, formal calculus, i.e., an axiomatic system containing first assumptions and derived conclusions, subject to the restraint of self-contained logic deducibility and freedom from contradiction. An essential aspect of such axiomatic systems is their property to contain certain terms to which, for the purpose of developing the calculus, no concrete meaning has to be attributed. Such prima facie undefined terms appear, for instance, in Euclid's geometry as "points," and many more examples could be adduced by way of illustration. I believe that also "probability" can be viewed as a point in case, and I therefore contend that the entire calculus of probability can be developed in its present form without assigning to this term any concrete meaning, i.e., a hypothetic or demonstrable entity in the real world.

Granting this approach, it becomes pertinent to inquire whether, in the real world, there exists a kind of events, or relationship between events, to which the formal calculus of probability stands in an isomorphic relation, meaning, thereby, that to each deduction in the calculus a uniquely determined type of event, or of relation of events, can be coordinated. Should the demonstration of such unique correspondence be successful, the meaning for the term "probability," undefined within the calculus, would emerge as the invariant common to all legitimate (i.e., successful) domains of application of the calculus. Now, the governing characteristics of all fields in which probability calculus is being applied comprise statements which, although having a rigorous form, have less factual content than an assertion of a certain fact would have and at the same time assert more factual content than a statement of complete ignorance would have. This applies to the games of chance which historically constituted the strongest impetus for the development of a calculus capable of predicting future events from a priori or a posteriori knowledge concerning a limiting ratio of success to failure in an entire aggregate of events, past *and* future; it equally applies to the evanescence of a measurement of a constant of Nature, in which case incompleteness of knowledge appears in the form of error; and it may be construed as the degree of confirmation in inductive reasoning with degrees of uncertainty required to assume rigorous, (numerical) expression. In all these instances, uncertainty of knowledge is subject to formulation in the same axiomatic frame, namely, the calculus of probability, with "probability" standing for a kind of relation between events which allows for, and gives precise expression to, uncertainty of knowledge. It is, I believe, this intricate and intimate union between a formal deductive system (i.e., probability calculus), carrying by its very nature the label of rigor and irrevocability of conclusions, and the calculation of risks of statements being wrong and, therefore, open to future falsification in which reside the special flavor of applied statistics and the pitfalls in its comprehension.

Whereas incompleteness of knowledge has so far been viewed as attribute of subject matters of inquiry (i.e., games of chance) and while uncertainty appeared as result of the class of all possible observations being open toward the future (error and induction), uncertainty and incompleteness of knowledge also found acceptance in some domains of inquiry as an explanatory high level hypothesis. When Maxwell implicitly employed, and Boltzmann and Gibbs explicitly pronounced, the notion that no system (as exemplified by

the initial state of a gas aggregate of particles) is random in itself but can be viewed as random by becoming a member of an ensemble (set) of analogous systems in which it loses its identity, testable laws could be derived from the application of probability calculus to infinite (hypothetic) sets of such systems. Here, then, uncertainty as a condition for applying probability calculus was introduced in the form of a first explanatory assumption (or hypothesis) from which were derived generalized predictions in the form of laws of Nature. In this case, renouncement of identifiability of individual members in hypothetic sets of such members, and consequent uncertainty and incomplete knowledge, constitutes, as an epistemic principle, a prerequisite for application of probability calculus. Thus, probability emerges as a condition for, and principle of, predictive *and* explanatory knowledge. The same basic conceptual and formal approach has more recently been extended into the field of information theory.

While the thermodynamics and statistical mechanics of Boltzmann and Gibbs required the invention of ensembles of systems in order to make probability calculus an applicable instrument, quantum mechanics had recourse to the "uncertainty principle" of Heisenberg as a necessary condition for a probabilistic approach to microphysical events.

Abstract as this way of thinking may appear, its impact on science and technology is very real: it replaced the physics of the fulcrum and the lever, and an economy of steam engines, by the physics of the electron cloud and the tools of the communication engineer.

If it is correct that the formal apparatus of probability calculus becomes applicable whenever an element of incomplete knowledge prevails—either as an ontologic principle pertaining to the subject matter of inquiry or as an epistemic principle governing the observer object relation—it is now incumbent on me to extend the argument to biologic experimentation and conclusions derived from it. It is not without interest that Democritus proposed to explain resemblances and dissimilarities between parents and children in terms of—as we would put it now—random arrangements of the atoms originating from ancestors. Darwin's recognition of the significance of variation in biology received its theoretic and experimental basis from modern genetics. In general, the possibility to predict from first assumptions relative frequencies with which definite characters occur in groups of individuals—assumptions which renounce knowability of precisely what gene combinations out of a set of possible ones will, in fact, occur—clearly demonstrates the applicability and success of probabilistic reasoning. When Clark and Gaddum revealed that the response of simple biologic test systems to drugs can be adequately accounted for by a statistical distribution of thresholds of sensitivity in a set of individual receptors, each one an inferred and unidentified entity, another successful avenue for probabilistic reasoning in biology was opened.

Biologic exploration usually involves study of complex phenomena of which only certain limited manifestations are accessible to direct measurement; explanation in this context amounts to invention of models from which statements can be deduced. The validity of the model is estimated from the closeness of fit between deductions from the hypothetic model and actual observations. These models, in the majority of instances, were found to require some element of indeterminism in order to satisfy the observational requirements. Such stochastic models are useful in a variety of fields, such as population growth and dynamics, life expectancy, natural selection, spread of epidemics, learning, and discharge characteristics of neuronal elements, individually or in aggregates. Less conspicuous, but equal in kind and importance, is the hypothesis underlying most studies of drug effects in populations, i.e., the hypothesis of a normal distribution of responsiveness. More generally, measurement in populations cannot be conducted

unless a specific hypothesis is formed which determines the distribution of the quality to be measured in an infinite ensemble of members of which the experimentally accessible population is a random sample.

To this kind of uncertainty in biologic experimentation and explanation (i.e., model construction) must be added the limitation of knowledge which arises from error of actual measurement. Thus, it appears that uncertainty enters the subject matter of biology in a twofold form: as observational error and in the form of hypothesis on elementary events.

The fact that one and the same probabilistic calculus applies equally to both kinds of limitation of knowledge should not lead to neglect of awareness of that fundamental distinction.

# Some effects of a new antiarrhythmic drug

*The effects of RO 2-5803, a compound shown to have antiarrhythmic properties in animals, were studied in 13 patients with atrial flutter or fibrillation. After a single oral dose, the atrial rate was slowed as much as 39 per cent; the effect lasted up to 24 hours. Intravenous administration produced similar effects with smaller doses.*

*After large oral doses, mild gastrointestinal disturbances were sometimes noted. Intravenous administration usually was followed by a transient, mild drop in blood pressure. One episode of severe hypotension occurred. There were no other untoward effects.*

*RO 2-5803 and quinidine caused similar slowing of the atrial rate. The drugs differed mainly in their effect on the ventricular rate. The cause of the ventricular slowing after RO 2-5803 administration is not clear. Further study is required to determine whether it is secondary to the slower atrial rate, because of direct depression of the A-V node or of a vagallike action.*

**Hyman R. Kahn, M.D.,* Daniel W. Lewis, M.D., John H. Killough, Ph.D., M.D., and William B. Abrams, M.D.** *Philadelphia, Pa.*
*Division of Cardiology, Department of Medicine, Jefferson Medical College*

The compound 2,6-bis(1-piperidylmethyl)-4-($\alpha$,$\alpha$-dimethylbenzyl)phenol dihydrochloride (RO 2-5803)† was demonstrated to have antiarrhythmic properties in isolated rabbit auricles and in acetylcholine-induced atrial fibrillation in dogs.‡ Equimolar concentrations of RO 2-5803, quinidine sulfate, and procaine amide slowed conduction velocity in isolated cat papillary muscle to approximately the same degree. RO 2-5803 was approximately twice as potent as quinidine sulfate in increasing the refractory time.

This report is concerned with the action of RO 2-5803 on the atrial rate in atrial flutter and fibrillation in man and compares it with quinidine sulfate. RO 2-5803 is shown to be effective in slowing atrial flutter or fibrillation but, as opposed to quinidine, to have no vagolytic action.

## Method

Thirteen hospitalized patients were studied. Eight had atrial fibrillation, three had atrial flutter, one had frequent premature atrial contractions, and one had normal sinus rhythm. One patient was treated first during an episode of atrial fibrillation and subsequently during an

Presented in part at the Sixty-first Annual Meeting of the American Therapeutic Society.

This investigation was supported in part by grants from Hoffman–La Roche Inc. and the Heart Association of Southeastern Pennsylvania.

Received for publication Aug. 16, 1960.

*Fellow in Cardiology, Heart Association of Southeastern Pennsylvania.

†Rhythmol.

‡R. A. Moe: Personal communication.

episode of atrial flutter. Thirty-six trials with RO 2-5803 and nine with quinidine sulfate were performed on the 13 patients. Except for the patient with normal sinus rhythm, all were taking digitalis. The dosage of digitalis and the type of diet varied. Initially RO 2-5803 was administered in single doses on successive days. Since in some cases drug effect was believed to persist for 24 hours, later experiments were spaced at least 48 hours apart. An infusion of 5 per cent glucose in water was begun in all patients receiving RO 2-5803 intravenously. The 50 mg. intravenous dose was diluted to 5 ml. and injected over a period of 4 minutes.

Before each experiment the patient rested in bed for at least 5 minutes. Blood pressure determinations were made at 1 minute intervals until stable, and then a single lead electrocardiogram was recorded. Immediately after this, the drug was administered, and thereafter electrocardiograms and blood pressure determinations were made at intervals of 1 minute to 2 hours.

Lead $V_1$ or precordial leads as described by Lewis were used to record rates. Patients whose electrocardiograms did not show atrial waves clearly were excluded. The rates were determined by counting at least 100 deflections. The ventricular rates were determined from electrocardiograms that were at least 1 minute long. All measurements were made by the same observer.

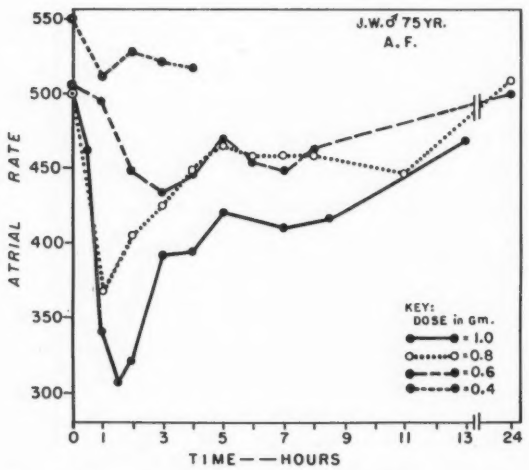



**Fig. 1.** The effect of single oral doses of RO 2-5803 on the atrial rate.

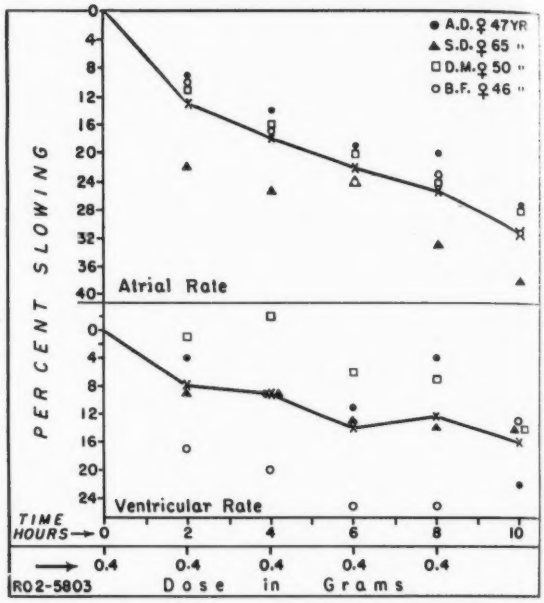



**Fig. 2.** Cumulative effect of repeated oral doses of RO 2-5803 on the atrial and ventricular rates in 4 patients with atrial fibrillation. The solid line represents the average effect in the 4.

## Results

### *Effect on atrial rate.*

*Oral doses.* Single oral doses of RO 2-5803 ranging from 0.2 to 1 Gm. were given a total of thirteen times to 5 patients with atrial flutter or fibrillation. Fifteen to 30 minutes after administration of the drug there was detectable slowing of the atrial rate. Maximum slowing usually occurred between 1 and 3 hours. During the next 1 to 3 hours, the effect decreased. It then remained constant for 6 to 9 hours before gradually returning to the control level by the end of 24 hours.

Fig. 1 illustrates the effect of RO 2-5803 on the rate in a 75-year-old man with arteriosclerotic heart disease and atrial fibrillation. The largest dose used in this case (1 Gm.) slowed the atrial rate by 193 beats per minute, 39 per cent of the control atrial rate.

The cumulative effects of repeated oral doses of RO 2-5803 was studied in 4 pa-

tients with atrial fibrillation and 3 with atrial flutter. These patients received 0.4 to 0.6 Gm. every 2 hours until five doses had been administered (total dose 2 to 3 Gm.). In 1 patient, atrial flutter was abolished and sinus rhythm restored approximately 4 hours after the last dose of RO 2-5803 (total dose 3 Gm.). The atrial arrhythmia persisted in the remaining patients. In all instances, however, there was a marked decrease in the atrial rate.

Fig. 2 shows the percentage of slowing in the 4 patients with atrial fibrillation who received a total dose of 2 Gm.

Electrocardiograms made before and after the administration of repeated doses of RO 2-5803 are shown in Fig. 3. There is marked slowing of atrial rate in all instances. The coarse atrial deflections used in counting the atrial rates are shown distinctly.

*Intravenous doses.* Single intravenous doses of 100 mg. were given to 2 patients with atrial fibrillation. The maximum effect was reached at 2 minutes in 1 patient and at 9 minutes in the other. The effect then gradually declined and disappeared in 2 to 3 hours.

Two patients with atrial fibrillation and 1 with atrial flutter were given 50 mg. of RO 2-5803 intravenously in repeated doses approximately 10 to 13 minutes apart. At this interval, a cumulative effect occurred. This is illustrated in Fig. 4. The maximum effect was seen within 2 minutes after each injection. Maximal slowing usually lasted about 9 minutes before beginning to wane.

***Effect on ventricular rate.*** After single oral doses, a slight decrease in ventricular rate was sometimes noted but the change was probably not significant. However, when repeated oral doses were given, the ventricular rate slowed consistently.

Following single intravenous injections of RO 2-5803, the ventricular rate increased momentarily simultaneously with the drop in systolic blood pressure. When repeated intravenous doses were given, the increase in ventricular rate tended to disappear after continued injections (Fig. 4).

***Comparison with quinidine sulfate.*** Comparison of the response to single oral doses

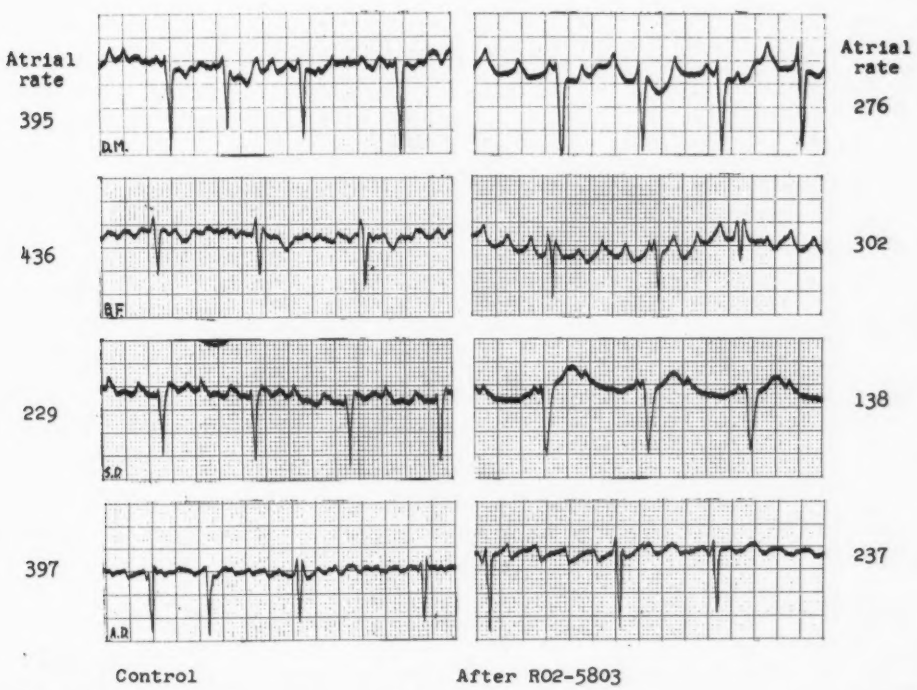



Fig. 3. Lewis leads taken before and after the administration of repeated oral doses of RO 2-5803. Atrial rate is shown to slow.

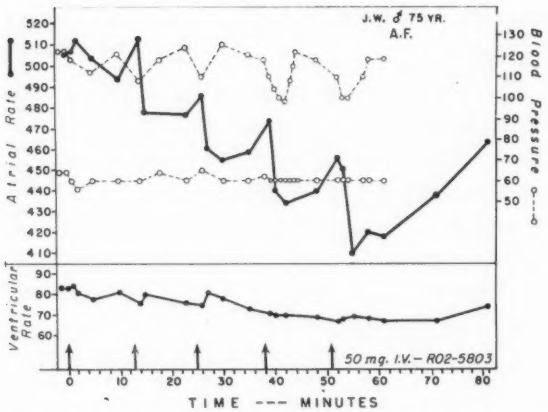



**Fig. 4.** Effect of repeated intravenous doses of RO 2-5803 on the atrial and ventricular rates.

of RO 2-5803 and quinidine sulfate was made in 2 patients with atrial fibrillation. In the first patient, there was 23 per cent slowing of the atrial rate after 1 Gm. of RO 2-5803 and 19 per cent after 0.4 Gm. of quinidine. In the second patient, 0.4 Gm. of quinidine sulfate and 0.8 Gm. of RO 2-5803 each slowed the atrial rate by 23 per cent.

In 3 patients, the effects of larger doses of quinidine sulfate and RO 2-5803 were compared. In each instance 0.4 Gm. of quinidine sulfate was given orally every 2 hours until a total dose of 2 Gm. had been given. On another day, similar doses of RO

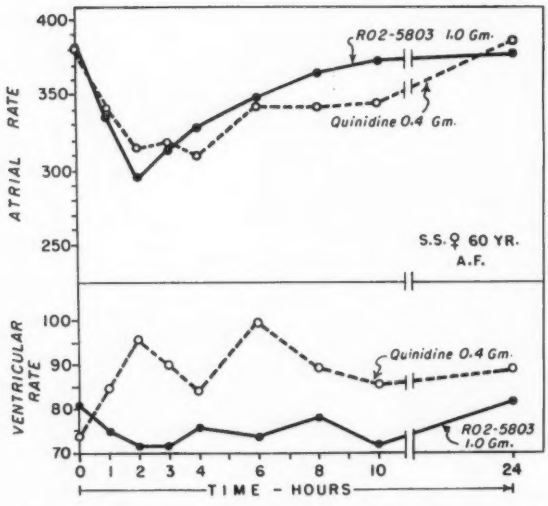



**Fig. 5.** Comparison of effects of quinidine and RO 2-5803 on atrial and ventricular rates.

2-5803 were given. The results are summarized in Table I; quinidine appeared to be the more effective.

The ventricular rate did not change appreciably after single oral doses of RO 2-5803 but rose after quinidine administration (Fig. 5). In all instances in which repeated doses were compared, the ventricular rate increased after quinidine and fell after RO 2-5803 ingestion.

***Untoward effects.*** All patients commented that the tablets (uncoated) of RO 2-5803 were bitter. One patient experienced nausea and vomited after a single dose of 0.8 Gm. but had no reaction to smaller doses. A second patient had nausea and vomited after a single dose of 1 Gm. With smaller doses she had transient nausea that was promptly relieved by the ingestion of food. A third patient vomited 15 minutes after the last dose of the 2 Gm. course. When given a total of 3 Gm. at a later date, she complained of lower abdominal cramps and diarrhea. The untoward effects with intravenous medication were limited to blood pressure changes. An 81-year-old man with arteriosclerotic heart disease and chronic atrial flutter had a drop in blood pressure from 150/70 to 100/0 mm. Hg and developed signs of peripheral vascular collapse after the fourth intravenous dose of 50 mg. (total 200 mg.) of RO 2-5803. The blood pressure promptly rose when 4 ml. of 0.2 per cent levarterenol bitartrate solution was added to the infusion and in 5 minutes was 175/98. Prior to the last injection, the ventricular rate had decreased from 56 to 38 beats per minute and did not rise with the fall in blood pressure. In all other cases, a transient drop in systolic blood pressure occurred within 2 minutes after intravenous administration. There was no change in diastolic pressure. No effect on blood pressure was observed following oral administration of RO 2-5803.

## Summary and conclusions

These experiments were designed to determine in patients the effect of RO 2-5803 on the atrial rate in flutter or fibrilla-

Table I

| Patient | Drug* | Atrial rate | | Change (%) | Untoward effects |
|---|---|---|---|---|---|
| | | Before | After | | |
| 1 | RO 2-5803 | 406 | 293 | −28 | None |
| | Quinidine | 400 | 229 | −43 | None |
| 2 | RO 2-5803 | 391 | 287 | −27 | Vomiting |
| | Quinidine | 358 | 198 | −45 | Nausea and diarrhea |
| | Quinidine | 398 | 230 | −42 | None |
| 3 | RO 2-5803 | 263 | 202 | −23 | None |
| | Quinidine | 248 | 185 | −25 | Weakness, anorexia, nausea |

*Dose of 0.4 Gm. every 2 hours to a total of 2 Gm. in each instance.

tion. Patients with *chronic* arrhythmias were chosen deliberately in the hope that conversion would not occur and a fairly complete dose response curve could be constructed. This aim was only partially achieved.

The smallest oral dose that produced slowing of the atrial rate was between 200 and 400 mg. The relative sizes of oral and intravenous doses necessary to produce similar effects in the same patient suggest that intestinal absorption is not complete.

RO 2-5803 and quinidine sulfate caused quantitatively equal slowing of atrial rate in flutter or fibrillation. They differed mainly in their effect on the ventricular rate. In every case, the ventricular rate increased with quinidine. This is the usual response and is ascribed to the vagolytic action of quinidine.[1, 2, 5] It occurs even in patients under complete digitalization.[4] Lewis was the first to emphasize that before administering quinidine in atrial fibrillation, digitalis should be used to slow the ventricular rate.[3] In contrast, the ventricular rate was not accelerated by RO 2-5803. This suggests that RO 2-5803 has no vagolytic activity. It is possible that atrioventricular nodal depression also assumes a role. This may be the result of direct action on the junctional tissues or of parasympathomimetic activity of the drug.

### *References*

1. Carr, J. G., and Spoeneman, W. H.: The untoward effects of quinidine, Illinois M. J. **46**: 445-449, 1924.
2. Hiatt, E., Brown, D., Quinn, G., and Macduffie, K.: The blocking action of the cinchona alkaloids and certain related compounds on the cardioinhibitory vagus endings of the dog, J. Pharmacol. & Exper. Therap. **85**:55-60, 1945.
3. Lewis, T.: The value of quinidine in cases of auricular fibrillation and methods of studying the clinical reaction, Am. J. M. Sc. **163**:781-794, 1922.
4. Lewis, T., Drury, A. N., Wedd, A. M., and Iliescu, C. C.: Observations upon the action of certain drugs upon fibrillation of the auricles, Heart **9**:207-267, 1922.
5. Nathanson, M. H.: Modification of vagus inhibition of heart by quinidine, Proc. Soc. Exper. Biol. & Med. **31**:1234-1236, 1934.

# The effect of psychopharmacologic agents on behavior, measured by the interaction chronograph

*This study is concerned with the objective measurement of drug-induced changes in interview behavior by means of the interaction chronograph, a device which allows an observer to record in time units with a high degree of precision the behavioral interaction of two individuals during a standardized interview in which the interviewer follows certain predetermined rules of interviewing. The background and details of the method are described. Sixty new patients, selected by chance from the Washington University Psychiatry Clinic, were given one of three possible drugs in a double blind procedure. They were subjected to a test-retest interview, using a standardized interview as a research tool and the interaction chronograph as the measure of response. Analysis of the data revealed statistically significant difference in the chlorpromazine group and no significant difference in the phenobarbital and placebo groups. The limitations and possibilities of the method are discussed.*

**Vicente B. Tuason, M.D., and Samuel B. Guze, M.D.** *St. Louis, Mo.*
*Department of Psychiatry and Neurology, Washington University School of Medicine*

Previous investigations have indicated that the interaction chronograph has promise as an instrument for assessing changes in behavior.[5, 6, 9, 10] The present study is a part of an endeavor to extend our understanding of the possibilities and limitations of the use of the interaction chronograph in the study of test-retest changes in behavior as a function of the administration of a drug.

In a recent double blind study of 50 patients,[4] we reported on the effect of three different doses of chlorpromazine (50 mg., 100 mg., and 150 mg.), 50 mg. of phenobarbital, and a placebo upon five groups of patients, using interaction chronograph measurements as the method of assessing the behavioral responses among the subjects. We reported finding no significant differences between any groups. Likewise, we did not find any significant differences when the subjective responses of the patients were compared.

It was decided to repeat this study with a larger sample and to limit the drugs used to one dose of chlorpromazine, 150 mg., 50 mg. of phenobarbital, and a placebo.

The interaction chronograph is essentially a device which allows an observer to record in time units with a high degree of precision the behavioral interaction of two

This investigation was supported by a research grant (MY-2242) from the Institute of Mental Health, U. S. Public Health Service.

Received for publication Sept. 14, 1960.

individuals in terms of some ten or more variables. A complete description of the variables studied and the method of observation can be found elsewhere.[6] A detailed review of the literature and a history of the development of the interaction chronograph and the theory underlying it are provided in a previous report.[1] The method ignores the content of the communications during interviews.

In his early studies with the interaction chronograph, Chapple used an unrestricted, nondirective interview. He later discovered that every interviewer was different not only in the way he behaved but in the results that he obtained from the same subject. Several reliability studies reported by him and Goldman-Eisler, working independently in England, confirmed the observation that the interviewer is not an objective instrument.[2, 3] They demonstrated that interviewers are themselves dependent, in the sense of uncontrolled, variables. Chapple therefore suggested some rules to guide the interviewer's behavior. The characteristics of the standardized interview and the "rules" governing the interviewer's behavior can be found elsewhere.[6]

The reliability of the various aspects of the standardized interview has been reported in earlier studies. These studies determined the observer,[7] scorer,[10] interviewer,[8] and interviewee reliability.[5, 9, 10]

The standardized interview involves complex behavior patterns on the part of the interviewer. The present study concerned itself with the simpler subperiod of the interview. In this, period I or the "free period," the interviewer tries to make his utterances as nondirective as possible, approximately 5 seconds long each time, without either interrupting the subject or delaying his response more than half a second. The reliability of this period and the number of observations necessary to achieve reliability have been established in a recent study.* The high correlations obtained in this reliability study comparing two 30 minute period I type interviews indicated the usefulness of this method for studying patient behavior during a standardized interview situation. The study demonstrated that using interaction chronograph measures, a 10 minute period of observation would be sufficient to reach a stable pattern of patient communication under period I conditions. It further established that a 30 minute period of observation would offer more stabilized interaction.

*Submitted for publication.

### Method

The research design called for interviewing 60 white persons selected as they presented themselves by chance to the Washington University Psychiatry Clinic. They were all either new patients in the clinic or had not been seen for at least a year. They were not receiving any medications. There were 14 men and 46 women ranging in age from 15 to 67. The presenting problems were typical of the outpatient clinic population. Diagnostically, there were 7 cases of anxiety reaction, acute and chronic, 11 cases of hysteria (conversion reaction), 8 cases of depression of the manic-depressive variety, 2 cases of normal reactive depression; 1 case of schizophrenia, 14 cases of personality disturbances (including 6 that fell under the category of sociopathic personality disturbance), 1 case of chronic brain syndrome, 3 cases of chronic alcoholism, 1 case of obsessive-compulsive neurosis, 11 cases of undiagnosed psychiatric disorder, and 1 case without any obvious clinical psychiatric difficulty.

Two experienced interviewers alternated in interviewing and observing each subject. The interviews were conducted on one side of a one-way vision screen, with the observer on the other side to activate the apparatus. The apparatus used to record the responses is an alternative to Chapple's machines. It consists of a five channel Esterline-Angus recorder using standardized charts that come ruled in various time

intervals. By selecting an appropriate gear ratio, the paper can be made to move 3 inches per minute. We used chart No. 1705-C, which provided ruled lines at 2 second intervals, with heavier lines at 10 second intervals and still heavier lines at 60 second intervals. These lines are $\frac{1}{10}$ inch, ½ inch, and 3 inches apart, respectively. The ink lines can be read to the nearest second. The record, using this apparatus, consists of three continuous synchronous parallel ink tracks, one for the patient, one for the interviewer, and a signal track. The appropriate pen is deflected about $\frac{1}{10}$ inch whenever the corresponding key on a small key box is depressed by the observer to indicate that the designated individual is interacting during the interview. At the conclusion of the interview, the relevant chronograph variables are extracted from the record: the number of times the patient interacts (units), the duration of the interaction (action), and the duration of the silence (silence).

Each patient was subjected to an initial 30 minute interview of period I type behavior. This served as the base line level. At the conclusion of this interview, the patient was given a capsule containing one of three possible ingredients (see below). Each subject was instructed to wait in an adjacent lounge and was directed not to take anything orally during the waiting period. After a period of 90 minutes, each subject was reinterviewed for another 30 minutes of period I type behavior. This observation period provided the drug effect level. The capsules were kept in individual, consecutively numbered envelopes which were used in order. The capsules, which were identical, contained either 150 mg. of chlorpromazine, 50 mg. of phenobarbital, or a placebo. The experiment was double blind in that the three groups of capsules containing different ingredients were selected by a table of random numbers and a colleague, who was not participating in the study, assigned the appropriate numbers.

**Table I.** *Ranges in interaction chronograph scores in each group studied*

| | *First interview* | | *Second interview* | |
|---|---|---|---|---|
| *Drug group* | *Units 1* | *Action 1 (sec.)* | *Units 2* | *Action 2 (sec.)* |
| Chlorpromazine (N = 20) | 2-74 | 19-993 | 1-127 | 11-1,800 |
| Phenobarbital (N = 20) | 2-60 | 24-913 | 5-51 | 27-402 |
| Placebo (N = 20) | 2-53 | 27-998 | 1-43 | 36-180 |

### Statistical analysis

The Wilcoxon Matched-Paris Signed-Ranks Test was used to analyze the data. This test provides for the consideration of the relative magnitude as well as the direction of the difference between the two interviews. It gives more weight to a pair which shows a large difference between two conditions than to a pair which shows a small difference.[11]

All statistical analyses were derived from the action (time duration in seconds subject interacts) and the units (number of times subject interacts) scores. The "silence" scores were not subjected to statistical treatment because of their limited range, thereby making the analysis of little usefulness. The circumscribed duration of the silence variable is due to the character of the interview itself.

### Results

For the entire sample, the units (frequency of responses) ranged from 2 to 74 in the first interview and 1 to 127 in the second interview. The mean action (average duration of time in seconds subject interacted) for the whole series ranged from 19 to 998 seconds in the first interview and 11 to 1,800 seconds in the second interview. The ranges in units and action scores for each drug group are shown in Table I. Because of the character of the interview, the subjects can vary their behavior so that the length of the action period may range from 1 to 1,800 seconds—

the full 30 minutes. In the accompanying table, action 1 and action 2 indicate the mean duration time in seconds a subject interacted in the first and second interviews, respectively. Units 1 refers to the number of times a subject initiated action during the first interview, and units 2 refers to the same measure for the second interview.

The results with the chlorpromazine group revealed that 17 out of 20 subjects prolonged the length of their action period and decreased their frequency of responses subsequent to ingestion of the drug. Application of the Wilcoxon Matched-Paris Signed-Ranks Test to the mean action scores indicates that this difference is significant at the 0.05 level of confidence (for $N = 20$, $p < 0.05$, $T = 52$). The difference failed to attain statistical significance at the 0.02 level by only one critical value of T (for $N = 20$, $p < 0.02$, $T = 43$). The results in the units scores revealed a difference significant at the 0.01 level.

In the phenobarbital-treated group, of 20 subjects, 9 decreased their duration of interaction, 9 increased the length of their action responses, and 2 did not change. The units scores show that 11 out of 20 subjects decreased their number of responses, 8 of the 20 subjects increased their units, and 1 obtained similar units in the two interviews. These findings are not statistically significant.

In the placebo group, 10 subjects increased and 10 subjects decreased the length of their action responses. In the units scores, 10 subjects increased their frequency of response, 8 decreased their number of responses, and 2 obtained tied scores. These scores are not significant statistically.

## Discussion

Our results show that within certain limits, this method permits discrimination between different drugs having an effect upon behavior. The negative results reported in our first study[4] may properly be attributed to the small samples used. It is clear from this study that the interaction patterns vary normally from one interview to the next and that the method requires a certain minimum size sample (approximately 20) to demonstrate significant differences. Further, the limits of sensitivity of the method are still not defined. We do not know yet how small a dose of chlorpromazine can be distinguished by the method, nor do we know the smallest dose of phenobarbital that the method will demonstrate.

We have not fully investigated as yet the various dose-time curves for different drugs. In our studies thus far, we have limited ourselves to responses that occur within 2 hours after the ingestion of the drug, because of the convenience in using outpatients (who will not wait much longer). At other intervals and with other routes of administration, drug effects may be more striking. Further study to determine the optimal times and dosages for various drugs are contemplated.

The chief value of the method, besides its quantitative nature, lies in its similarity to normal clinical interviewing. This permits the same subject to be studied repeatedly, under various circumstances, in a situation that appears to him to be a normal clinical one. The subject is unaware of the nature of the observation and experiment and so may be used for many different comparisons.

### *References*

1. Chapple, E. D.: The interaction chronograph, its evolution and present application, Personnel **25**:295-307, 1949.
2. Chapple, E. D.: The standard experimental (stress) interview as used in interaction chronograph investigations, Human Organization **12**: 23-32, 1953.
3. Goldman-Eisler, F.: Individual differences between interviewers and their effect on interviewee's conversational behavior, J. Ment. Sc. **98**:600-670, 1952.
4. Guze, S. B., Tuason, V. B., McClure, J., and Beguelin, J.: The effect of chlorpromazine on behavior, Clin. Res. **8**:175, 1960.
5. Matarazzo, J. D., Saslow, G., and Guze, S. B.: The stability of interaction chronograph pat-

terns in psychiatric interviews: A replication, J. Consult. Psychol. **20**:267-274, 1956.

6. Matarazzo, J. D., Saslow, G., and Matarazzo, R. G.: The interaction chronograph as an instrument for objective measurement of interaction patterns during interviews, J. Psychol. **41**:347-367, 1956.
7. Phillips, J., Matarazzo, J. D., Matarazzo, R., and Saslow, G.: Observer reliability of interaction patterns during interviews, J. Consult. Psychol. **21**:269-275, 1957.
8. Saslow, G., and Matarazzo, J. D.: A technique for studying changes in interview behavior, *in* Research in psychotherapy, Washington, D. C., 1959, American Psychiatry Assn., Inc., p. 125.
9. Saslow, G., Matarazzo, J. D., Phillips, J. S., and Matarazzo, R. G.: Test-retest stability of interaction patterns during interviews conducted one week apart, J. Abnorm. & Social Psychol. **54**:295-302, 1957.
10. Saslow, G., Matarazzo, J. D., and Guze, S. B.: The stability of interaction chronograph patterns in psychiatric interviews, J. Consult. Psychol. **19**:417-430, 1955.
11. Siegel, S.: Non parametric statistics for the behavioral science, New York, 1952, McGraw-Hill Book Company, Inc., pp. 75-83.

# Further studies on the action of chlorpropamide in diabetes mellitus

*Diabetes mellitus of moderate severity in adult patients was regulated with chlorpropamide in decreasing dosage. The level of regulation of diabetes remained unchanged until the dosage was decreased below a critical minimum or was omitted entirely. After a period of elevation of blood sugar for from 23 to 63 days, regulation of diabetes was attempted with the lowest previously effective dosage, but it frequently failed. The significance of this observation is discussed.*

**Samuel B. Beaser, M.D.*** *Boston, Mass.*
*Harvard Medical School and the Medical Service of Beth Israel Hospital*

This article presents further data on a previous study of the relation between oral dosage, blood levels, and clinical and metabolic activity of chlorpropamide† in the treatment of adult diabetes mellitus. The plan of the first study[1] included the gradual decrease in dosage of chlorpropamide in successfully treated adult diabetic patients over several months to determine the minimum effective dosage and blood level of the drug. It was observed that the drug often had an equal effect over widely varying dosage levels, down to 100 mg. per day. This is an attempt to explore this phenomenon further.

Six adult diabetic patients on chlorpropamide therapy were chosen for this study. Their diet and weight were constant. They each maintained a blood sugar level of 130 to 170 mg. per 100 ml. (Folin-Wu method) 1 hour after breakfast during several months of progressive decrease in chlorpropamide dosage.

### Method of study

Five of the patients were chosen for further reduction of dosage from 100 mg. to 50 mg. per day and the other, one with more severe diabetes mellitus (patient 6), for reduction from 250 mg. to 125 mg. per day (Table I). One of the former (patient 5, Fig. 5) and patient 6 showed a distinct escape with a rise in blood sugar level while on the lowered dosage of drug. The medication of the remainder was finally discontinued entirely (Figs. 1 through 4). In all patients, the blood sugar levels were determined at 1 to 3 week intervals. After a persistent rise in blood sugar level had occurred, an attempt to reestablish regulation was made with the last effective dosage. The dosage was then raised successively every few weeks until the original blood sugar level was restored.

This study was supported by a grant from Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

Received for publication Nov. 7, 1960.

*Clinical Associate in Medicine; Consultant, Abraham Rudy Diabetes Clinic.

†Diabinese.

**Table I.** *Determination of chlorpropamide dosage adequate for restoration of diabetes regulation after withdrawal*

| | | | | | Chlorpropamide therapy | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Initial regulation | | | Period of blood sugar elevation | | | | Final regulation | |
| | | | | | | Dose adequate | | | | | | | |
| Pt. | Graph | Age (yr.) | Duration of diabetes (yr.) | Previous insulin (U. of NPH per day) | Duration (mo.) | Initial (Gm. per day) | Final (Gm. per day) | Dose (Gm. per day) | Earliest onset (days) | Maximum (days) | Total duration (days) | Dose inadequate (Gm. per day) | Dose adequate (Gm. per day) |
| 1 | Fig. 1 | 79 | 0.5 | | 1 | 0.1 | 0.05 | 0 | 12 | 48 | 63 | | 0.05 |
| 2 | Fig. 2 | 56 | 12 | 35 | 11 | 1.5 | 0.05 | 0 | 20 | 20 | 20 | 0.2 | 0.25 |
| 3 | Fig. 3 | 72 | 11 | 14 | 8 | 1.0 | 0.05 | 0 | 12 | 19 | 50 | 0.05 | 0.1 |
| 4 | Fig. 4 | 50 | 5 | | 11 | 1.0 | 0.05 | 0 | 13 | 13 | 23 | 0.2 | 0.25 |
| 5 | Fig. 5 | 33 | 7 | | 8 | 1.5 | 0.1 | 0.05 | | 30 | 30 | 0.5 | 0.5 plus phenformin |
| 6 | | 62 | 2 | 58 | 11 | 0.6 | 0.25 | 0.125 | 30 | 30 | 30 | 0.125 | 0.25 |

In 6 patients, diabetes of moderate severity was regulated with decreasing doses of chlorpropamide to blood sugar levels of 150 to 170 mg. per 100 ml. (Folin-Wu method) 1 hour after breakfast. Blood sugar levels were allowed to rise to or over 200 mg. per 100 ml. by inadequate dosage or omission of drug. Then the dose adequate for restoration of regulation was determined.

Blood sugar and blood chlorpropamide levels were determined 1 hour after breakfast or lunch. The former was performed by the Folin-Wu method and the latter by a spectrophotometric method.[8]

### Results

Table I shows that it took about 12 to 48 days for the blood sugar to reach a level of 220 to 250 mg. per 100 ml. during metabolic escape, with evidence of such a trend within 2 weeks in most patients.

After 23 to 63 days of an elevated blood sugar level, patients 2 through 5 did not revert to a normal blood sugar level after reinstitution of the dosage of drug successful just prior to the metabolic decompensation. In patients 1 and 6, this dosage did regulate the diabetes again. The former was a mild diabetic, and the latter had become decompensated while still receiving a substantial dosage of the drug. In patient 5, normoglycemia could not be restored by chlorpropamide alone, and addition of phenformin was required to effect return to the previous blood sugar level.[2]

The blood chlorpropamide levels reached at corresponding dosage levels of chlorpropamide were substantially the same before and after the hyperglycemic experience in all the patients. There was no evidence of alteration in metabolism or excretion of the drug to account for change in activity.

### Case reports

***Patient 1.*** This patient was a 79-year-old white woman with diabetes whose initial blood sugar level was 350 mg. per 100 ml. (Fig. 1). After the diabetes had been successfully regulated with 50 mg. of chlorpropamide per day for 60 days, the drug was discontinued. After 48 days, the blood sugar level rose to 250 mg. per 100 ml. On the sixty-third day, the last effective dosage of chlorpropamide (50 mg. per day) was readministered, and in 35 days the blood sugar level returned to the level prior to metabolic decompensation.

***Patient 2.*** A 56-year-old white man with diabetes of 12 years' duration had received insulin injections for 5 years, and required at first 16 U. and later 35 U. of NPH insulin (Fig. 2). He was also successfully treated with carbutamide and tolbutamide. Chlorpropamide was started with a dosage of 1.5 Gm. per day. During the next 329 days, the dosage of chlorpropamide was grad-

ually reduced to 50 mg. per day with no change in blood sugar level. The drug was then omitted, and polyuria, somnolence, and rise of blood sugar occurred within 20 days. Readministration of chlorpropamide at a dosage of 100 mg. per day was inadequate, and after 6 months of gradual increase in dosage to 250 mg. per day, the original blood sugar level was restored: a fivefold increase in drug requirement.

***Patient** 3.* This patient was a 72-year-old white man with diabetes of 11 years' duration who required 14 units of NPH insulin for regulation (Fig. 3). The diabetes had been successfully regulated with tolbutamide. Chlorpropamide was administered over an 8 month period in dosages progressively decreasing from 0.5 Gm. to 50 mg. per day. The blood sugar level remained essentially unchanged, at 130 to 160 mg. per 100 ml. The drug was then omitted, and within 3 weeks the blood sugar had risen to 200 mg. per 100 ml. After 50 days without treatment, chlorpropamide was resumed. A dosage of 100 mg. per day was required to restore the original blood sugar level.

***Patient** 4.* A 50-year-old white man with diabetes of five years' duration (Fig. 4) had been successfully treated with tolbutamide. Chlorpropamide, 1 Gm. per day, was substituted. Over the next 313 days, a blood sugar level of 150 mg. per 100 ml. was maintained despite decrease of the dosage of the drug to 50 mg. per day. The drug was then discontinued; after 23 days, the blood sugar level was 285 mg. per 100 ml. Therapy was resumed to 50 mg. per day, but 250 mg. per day was required to restore the original blood sugar level after a total of 167 days.

***Patient** 5.* A 33-year-old white man with diabetes of 7 years' duration had been on dietary treatment alone until 2 years before (Fig. 5). At that time, 2.5 Gm. of tolbutamide per day failed to regulate the diabetes. However, a blood sugar level of 150 to 160 mg. per 100 ml. was maintained over the next 8 months as chlorpropamide was administered in dosages decreasing from 1 Gm. to 100 mg. per day. When the dosage was decreased to 50 mg. per day, the blood sugar level rose to 230 mg. per 100 ml. After a 30 day period of hyperglycemia, chlorpropamide therapy was resumed. This proved unsuccessful over the next 275 days even with a dosage of 500 mg. per day, and it was necessary to add phenformin to restore the original blood sugar level.

***Patient** 6.* This patient was a 62-year-old white woman who had had diabetes for 2 years. At the onset, she had both an infected toe and acidosis, and she required 58 U. of NPH insulin. Then the diabetes was regulated successfully with a combination of tolbutamide and phenformin. Eventually, the diabetes was well controlled with 0.6 Gm. of chlorpropamide alone per day. Over the next 11 months, a constant blood sugar level of 160 to 170 mg. per 100 ml. was maintained even though the dose of chlorpropamide was gradually reduced to 250 mg. per day. When this was further reduced to 125 mg. per day, the blood sugar level rose to 220 mg. per 100 ml. After 1 month of hyperglycemia, regulation was restored by the original dose of 250 mg. per day.

## Discussion

In this group of patients, a fairly steady state of diabetic regulation was effected over the entire therapeutic range of chlorpropamide dosage and blood levels. The most likely inference is that this represented the maximum response of the target of the sulfonylurea drug, namely, the pan-

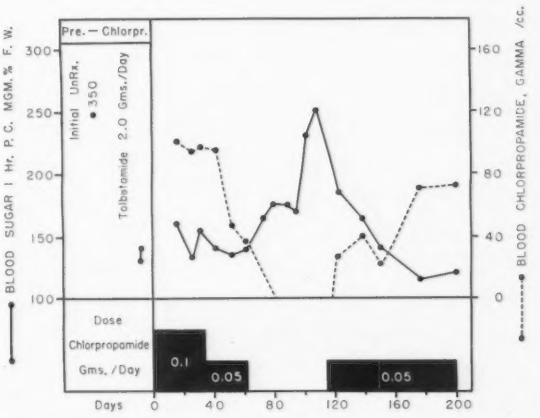



**Fig. 1.** Patient 1, a 79-year-old woman with diabetes which was regulated on 50 mg. of chlorpropamide per day, decompensated when drug was withdrawn, and again regulated on the same dosage.

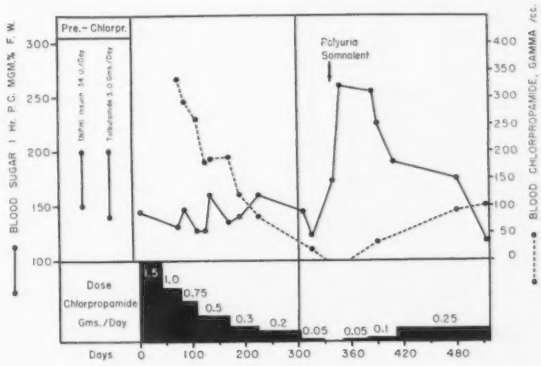



**Fig. 2.** Patient 2, a 56-year-old man with diabetes which was regulated on 50 mg. of chlorpropamide per day, decompensated when drug was withdrawn, and required 250 mg. per day to be regulated again.

creatic beta cell tissue. This is supported by the observation that a relatively constant response also may occur when increasing dosage of the drug is administered to a responsive patient (Fig. 6). On the other hand, it might be inferred that there is an increase in effectiveness of the sulfonylurea drugs with time in some of the patients. Occasionally, patients are indeed encountered who require larger dosages of these drugs for an optimum blood sugar response within the first 2 months but later have the same blood sugar levels on reduced dosage.

The speed of metabolic decompensation was relatively slow after critical reduction or omission of the drug. This has also been observed by others and has been attributed to an ameliorating effect of the sulfonylurea drugs upon diabetes.[6, 7] This may be a characteristic of adult diabetes, however, rather than a specific effect of the drug, since it can also occur after insulin withdrawal.

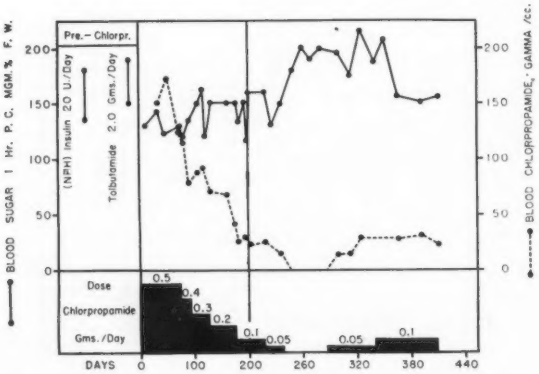



**Fig. 3.** Patient 3, a 72-year-old man with diabetes which was regulated on 50 mg. per day of chlorpropamide, decompensated when drug was withdrawn, and required 100 mg. per day to be regulated again.

Five patients did not repeat their original response to the drug after a period of blood sugar elevation above 200 mg. per 100 ml. This could not be attributed to a too short period of readministration or to any alteration in the metabolic fate of the drug after the hyperglycemic episode. The blood chlorpropamide levels attained at a given dosage of the drug were adequate during its readministration.

This state of refractoriness may not be permanent. Patients 2 and 3 were able to return to the original lower dosage at a later date. The patient with striking secondary failure of action (patient 5) still requires added phenformin for proper regulation.

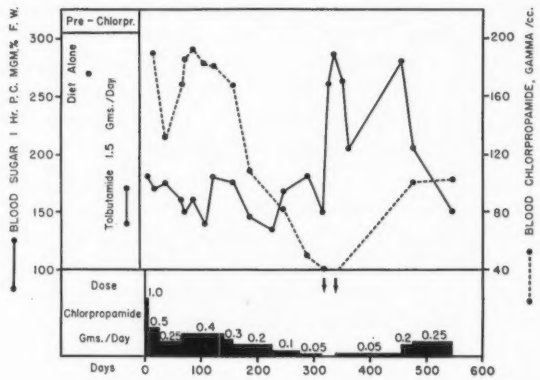



**Fig. 4.** Patient 4, a 50-year-old man with diabetes which was regulated on 50 mg. per day of chlorpropamide, decompensated when drug was withdrawn, and required 250 mg. per day to be regulated again.

Increase in severity of diabetes as measured by increased insulin requirements has been described after a variety of stressful situations such as diabetic acidosis, infection, and myocardial infarction, and it has followed periods of poor diabetic regulation. It is also seen during the initial therapy of new diabetic patients who have elevated blood sugar levels. It may be either temporary or permanent, and its cause may be extrapancreatic.[4, 5]

The acquired resistance to the drug is probably not due to simple interruption of sulfonylurea therapy. A previous article[3] reported on a group of 8 patients in whom, after successful therapy with tolbutamide, diabetes was regulated with insulin. The period of interruption was 8 to 11 days, but the blood sugar levels maintained by insulin in the interval had been relatively

normal. In three of those patients, immediate regulation with tolbutamide with no loss of effectiveness of the drug was accomplished.

Certain practical clinical implications can be drawn from these data. It would appear unwise to withdraw sulfonylurea therapy in patients successfully treated with it, without close follow-up. Since some of these patients were asymptomatic during the period of hyperglycemia, follow-up must include laboratory tests. This also applies to the interruption of therapy because of inability to ingest the drug, e.g., during nondiabetic complications, medical or surgical. Under those circumstances, prompt substitution of insulin is essential.

The new, untreated diabetic patient is known to have relative insulin resistance. Insulin activity is dose dependent and can be increased continuously to effect normoglycemia in all but the most insulin resistant patients. Sulfonylurea therapy, on the other hand, has a definite ceiling to its activity. Patients 2 to 6 of this group can be said to illustrate a comparison of the response to the sulfonylurea drugs between "regulated" and "unregulated" patients, respectively.

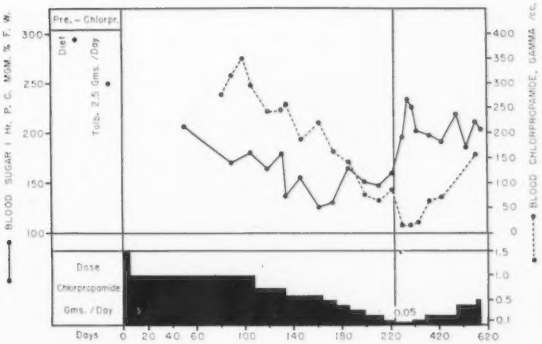



**Fig. 5.** Patient 5, a 33-year-old man with diabetes which was regulated on 100 mg. per day of chlorpropamide, decompensated on 50 mg. per day, and required both 500 mg. of chlorpropamide per day and phenformin to be regulated again.

These observations may provide the rationale for not starting therapy in recent, unregulated diabetes with minimal dosages of sulfonylurea drugs. In fact, they may also suggest not beginning the treatment of more severe diabetes with a sulfonylurea drug until after regulation has been accomplished with insulin. Time alone will not result in normoglycemia if the dosage of the sulfonylurea drug is too low or the severity of the diabetes is too great. These and similar data derived from a personal experience with chlorpropamide in over 250 cases of diabetes have led to the formulation of a therapeutic program for regulation of diabetic patients with this drug.

New diabetic patients are best started with a dose of 100 or 250 mg. of chlorpropamide once daily, depending on severity, as judged by blood sugar level and other criteria. If a new diabetic patient is under 30 years of age or has had severe symptoms, ketonuria, or a postprandial blood sugar level of over 300 mg. per 100 ml., initially regular insulin is preferable. Subsequently, chlorpropamide may be added, with gradual withdrawal of insulin. This is also the case in older insulin-treated diabetic patients with insulin requirements of over 20 U. in whom the dosage is assessed simply as that required to attain normoglycemia.

The evidence to date shows that practically all instances of significant toxicity

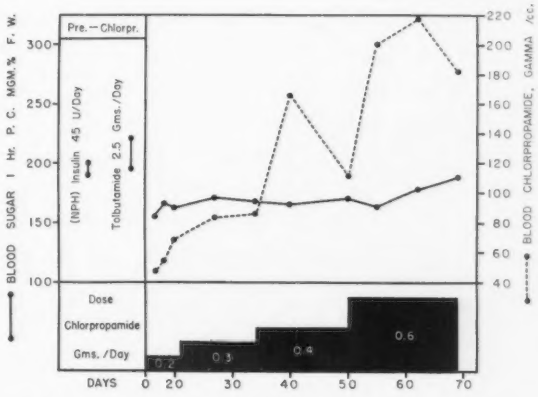



**Fig. 6.** Patient 6, a 73-year-old male diabetic whose blood sugar level remained at 150 to 180 mg. per 100 ml. while on a dosage of chlorpropamide increasing continuously from 200 to 600 mg. per day.

with chlorpropamide have occurred within the first 6 to 8 weeks of therapy, if a dosage level of 500 mg. per day or over is used during that period.* Accordingly, it is best to maintain a dosage of chlorpropamide below 500 mg. per day for the first 6 to 8 weeks, supplemented by insulin if necessary to maintain a normal blood sugar level. After 6 weeks, insulin can be withdrawn, and only then should the dosage of chlorpropamide be raised to 500 mg. per day in order to complete regulation. Equally useful to minimize toxicity is the procedure of adding phenformin to the regimen whenever more than 375 mg. of chlorpropamide appears to be necessary, whether at the beginning of treatment or later.[2]

### Conclusion

This is a continuation of previous studies on the relationship between dosage, blood levels, and clinical action of chlorpropamide.

In 6 adult patients, diabetes of moderate severity was regulated with chlorpropamide with a blood sugar level of 130 to 170 mg. per 100 ml., 1 hour after eating. The dosage of chlorpropamide was then reduced gradually over a period of several months, and repeated blood sugar determinations were made. The blood sugar level remained unchanged over a wide range of drug dosage, from 1.5 Gm. down to 50 mg. per day.

When the dose of chlorpropamide was reduced to a critical level in 2 or omitted entirely in 4 patients, the blood sugar level rose during 2 or more weeks. After 23 to 63 days of hyperglycemia, chlorpropamide was readministered at the last dosage which had proved adequate for maintenance of the steady and reduced blood sugar level. In 5 of the 6 patients, this proved inadequate and a higher dosage was required for regulation.

One of these 5 patients remained permanently refractive to chlorpropamide and required the addition of phenformin to complete regulation. Of the other 4, in 2 diabetes could be regulated on a lower dosage of drug after a long period of time.

The mechanism and clinical significance of this refractory state is discussed. In addition, a general outline of the clinical use of chlorpropamide is given, with special attention to the method of avoidance of toxic effects.

### *References*

1. Beaser, S. B.: The correlation between oral dosage, blood levels and clinical and metabolic activity of chlorpropamide in the treatment of diabetes mellitus, Ann. New York Acad. Sc. **74**: 701-708, 1959.
2. Beaser, S. B.: Therapy of diabetes mellitus with combinations of drugs given orally, New England J. Med. **259**:1207-1210, 1958.
3. Beaser, S. B.: Further experience with the use of sulfonylureas in diabetes, Ann. New York Acad. Sc. **71**:264-267, 1957.
4. Cahill, G. F. Ashmore, J., Renold, A. E., and Hastings, A. B.: Blood glucose and the liver, Am. J. Med. **26**:264-267, 1959.
5. Field, J. B., and Rigby, B.: Circulating insulin antagonists in diabetes mellitus: Untreated diabetics, diabetics during infections and acromegalics with diabetes, J. Endocrinol. **19**:174-181, 1959.
6. Loubatières, A.: General pharmacodynamics of the hypoglycemic arylsulfonamides, Ann. New York Acad. Sc. **74**:413-418, 1959.
7. Sugar, S. J. N., and Thomas, L. J.: Use of chlorpropamide in diabetes mellitus in usual diabetic patients and in primary and secondary tolbutamide failures, Ann. New York Acad. Sc. **74**:625-631, 1959.
8. Toolan, T. J., and Wagner, R. L., Jr.: The physical properties of chlorpropamide and its determinations in human serum, Ann. New York Acad. Sc. **74**:449-458, 1959.

*R. Warner, Chas. Pfizer & Co., Inc.: Personal communication.

# Circulatory actions of general anesthetic agents and the homeostatic roles of epinephrine and norepinephrine in man

*Circulatory actions of general anesthetic agents are viewed as resulting from three primary causes: (1) reduced metabolic demand resulting in turn from anesthetic depression of cellular activity throughout the body, (2) reduced functional ability of the heart and vasculature caused by direct anesthetic actions on these structures, and (3) autonomic nervous actions which modify cardiovascular regulation. Hemodynamic changes attributable to reduced metabolic demand represent normal homeostatic responses, but those caused by anesthetic depression of cardiovascular structures or reflexes tend to destroy homeostasis and are potentially lethal. It is significant that each of the four general anesthetics most commonly used in man has been found to disturb cardiovascular regulation, and it may be assumed that all such drugs possess side effects of this type. However, it is important as well as interesting that each of the drugs examined evokes a characteristic mixture of hemodynamic and autonomic actions which is distinct from that caused by any other. This suggests that circulatory side actions may ultimately be predictable from drug structure and that an ability to design anesthetics with desirable circulatory actions will prove worthy of cultivation.*

**Henry L. Price, M.D.,*** *Philadelphia, Pa.*
*Department of Anesthesiology, University of Pennsylvania Schools of Medicine*

A practical aspect of the condition known as "general anesthesia" is that the first word is just as descriptive as the second. No general anesthetic agents are known which act only on nervous tissues subserving sensation. They act in most or all body areas, including nonnervous ones, and it is this generality of action which is a principal concern of anesthetists.

It is for this reason that anesthetic drugs cannot be selected for clinical use solely on the basis of their anesthetic properties. Anesthetists recognize that "side effects," which frequently consist of circulatory disorders, are evoked simultaneously with the desired actions of the drugs administered. Frequently these side effects are so pronounced that they affect, or even dictate, the choice of anesthesia for a particular pa-

Work in the author's laboratory was supported in part by grants H 1568 ($C_6$) and H-4589 from the National Institutes of Health, U. S. Public Health Service, and by grants from the office of the Surgeon General, U. S. Army (DA-49-007 MD 599).

Received for publication Sept. 21, 1960.

*Wellcome Associate Professor of Research in Anesthesiology. Present address: Cardiovascular Research Institute, University of California Medical Center, San Francisco, Calif.

tient. Occasionally, death has resulted from these undesirable actions. In such cases, failure of the circulation has usually been the apparent cause of fatal outcome.

Circulatory actions of general anesthetics are of two major types: (1) those attributable to direct depression of cardiovascular function and (2) those caused by interference with autonomic reflexes. Direct actions on blood vessels appear relatively small at "light" depths of surgical anesthesia,[41] but no general anesthetic is known which, when present in an anesthetic concentration, fails to depress contractility in isolated mammalian hearts.[16, 22, 29, 32, 34, 60, 68, 77, 92, 95, 100] Moreover, cardiac depressant and anesthetic effects run parallel over a wide range of concentrations for all general anesthetics which have been studied, and while some anesthetics are more depressant than others, the general similarity between their over-all effects in the cerebrum and in the heart is striking.[7, 68] In addition, depressant effects on peripheral vascular beds become evident as the "depth" of anesthesia is increased.[41] It seems obvious, then, that the administration of a general anesthetic can produce circulatory failure unless its direct actions are antagonized homeostatically.

In this review, the role of the sympathetic nervous system in preserving circulatory homeostasis during general anesthesia in man is to be examined. It is reasonable to begin by asking whether epinephrine and norepinephrine, which are physiologically the most important products of sympathetic nervous activity, can correct the functional deficits caused by general anesthetics. The answer appears to be "yes with qualifications." For example, one or both catecholamines were found capable of antagonizing the reduction in cardiac contractile force and also the increase in mechanical impedance caused by various anesthetics,[1, 13, 21, 26] but they failed to restore relaxation rate to normal.[13] Similarly, dilatation in larger blood vessels the activity of which is conditioned primarily by neurogenic stimuli might readily be reversed by increased sympathetic nervous activity, while suppression of the intrinsic activity of the finest vessels[41] might be less successfully antagonized. Some idea of the potential importance of sympathetic nervous activity is gained from the observation that intravenous infusions of norepinephrine protected artificially respired dogs against as much as four times the dose of thiopental or pentobarbital which was lethal in untreated animals.[21]

In view of these findings, it is important to know how homeostatic reflexes fare during general anesthesia. If they were entirely normal, direct cardiovascular actions of anesthetic drugs might to a large extent be antagonized by the reflex release of epinephrine, norepinephrine, or both, and departures from normal circulatory function could be viewed as representing nothing more than responses to changing metabolic demand. Unfortunately the situation is not so simple. First, there is the embarrassing fact that virtually every relevant study of these reflexes has been performed in anesthetized animals! And second, in the typical analysis, only the pressor response to carotid occlusion is measured so that one cannot learn either the means whereby the reflex response is modified (site of drug action) or what the measured changes mean in terms of circulatory control. Neither is it known with certainty that the results of animal studies apply as well to man. However, it is probably safe to conclude from the available evidence that none of the drugs in clinical use fail to depress cardiovascular reflexes, at least to some extent. The relative potency of various anesthetic agents in causing depression appears to run, roughly (from most to least depressant), volatile anesthetics (chloroform, ether, halothane) > oxybarbiturates > cyclopropane > thiopental–nitrous oxide > chloralose.[2, 42, 55, 62, 79, 84, 94]

This ability of general anesthetic drugs to interfere with cardiovascular reflexes complicates the analysis of their actions remarkably, because it suggests that significant departures from normal circulatory

function may occur during anesthesia without calling forth adequate compensatory responses and, equally, that intense autonomic nervous activity might be elicited in the absence of any need for it.

The list of possible causes for circulatory alterations during general anesthesia must therefore be expanded. To altered tissue metabolic demands must be added various types of autonomic imbalance caused by anesthetic actions in diverse parts of the nervous system, direct cardiovascular depression which is not successfully antagonized reflexly, and a number of other departures from normal function (respiratory acidosis, for example) which result from anesthetic depression of other systems.

In this situation, the only way of attempting to determine whether circulatory homeostasis is preserved during anesthesia appears to consist of assembling all the data available and then of deciding, so far as possible, the significance of departures from normal. In the remainder of the review, this method of analysis will be pursued. Actions of the four general anesthetics most commonly used in man (cyclopropane, ether, halothane, and thiopental–nitrous oxide) will be discussed.

### Cyclopropane

Of all general anesthetics, the circulatory actions of cyclopropane have been most thoroughly studied. Both in normal men and in trained dogs, cardiac output and stroke volume were elevated during "light" anesthesia, returning toward or below normal with increasing cyclopropane concentration.[45, 81] Oxygen consumption was affected little; consequently, the arteriovenous oxygen difference was diminished except during "deep" anesthesia. Cardiac output was generally reduced by much lower concentrations of cyclopropane in patients who received morphine before the induction of anesthesia,[45, 49, 90] often returning to or toward normal with increasing duration of anesthesia.[45] The preanesthetic use of morphine makes analysis of the actions of cyclopropane needlessly complicated; in the remainder of this review, the results of simpler studies will be emphasized whenever data are available.

Most authors agree that mean arterial pressure is usually elevated during cyclopropane anesthesia,[45, 49, 67, 90] and when cardiac output approaches or falls below normal (i.e., in light to moderately deep cycloprapane anesthesia or light anesthesia after morphine), total peripheral resistance is consequently increased. Areas of increased vascular resistance during deep anesthesia include the kidney,[18, 37] liver,[37, 88] and (to a lesser degree) the forearm.[47] In most of these studies, efforts were made to exclude hypoventilation, positive pressure lung ventilation, and surgical stimulation as causes for the circulatory changes observed. Presumably they resulted from pharmacologic actions of cyclopropane.

In man the concentration of norepinephrine in arterial plasma, when measured by suitable techniques, was found to increase markedly during cyclopropane administration.[69, 72, 73, 93] This finding suggests increased sympathetic nervous activity in the body. Infusion experiments have shown that the increase in plasma norepinephrine concentration caused by cyclopropane administration can be duplicated by infusing norepinephrine intravenously at a rate of 5 to 10 $\mu$g per minute.[72] Analysis of adrenal venous blood and studies of adrenalectomized subjects indicated that the adrenal glands were not the source of the increased plasma concentration of norepinephrine.

Likely sources for the increased quantity of norepinephrine in plasma appear to be sympathetic nerves supplying the viscera. It has been shown in dogs and cats that when sympathetic nerves supplying the heart,[64] liver,[53] intestine,[54] spleen,[53] or uterus[54] are stimulated electrically, norepinephrine is liberated into the effluent blood. It is important to note that the rate of stimulation required to release norepinephrine into blood appears high in relation to the range of impulse frequencies normally tra-

versing these nerves.[10, 11] In other words, norepinephrine does not appear in blood leaving the viscera unless these organs are subjected to marked sympathetic stimulation, and the appearance of norepinephrine in plasma during cyclopropane anesthesia consequently suggests that relatively intense sympathetic nervous activity is present. The sympathetic mediator normally fails to appear in venous blood because it is largely destroyed in situ after combination with tissue receptors.[11] But even the small amount which escapes destruction in the tissues and enters venous blood during cyclopropane anesthesia (5 to 10 μg per minute) is sufficient to cause conspicuous hypertension (20 to 40 mm. Hg) when infused intravenously into normal human subjects.[72, 97] Thus, we have reason to regard the increase in plasma concentrations of norepinephrine during cyclopropane administration as evidence of a physiologically important activity.

Does this activity account for increased vascular resistance, increased stroke volume, and arterial hypertension during cyclopropane administration? And does it account for the fact that cardiac contractile force is said to be fairly well maintained in man during cyclopropane anesthesia,* while in the isolated heart myocardial competence fails rapidly on exposure to cyclopropane?[60, 68] In both cases the answer may well be "yes." In man profound hypotension, rather than hypertension, was produced by cyclopropane in subjects who were "chemically sympathectomized" (by 30 ml. of 0.1 per cent procaine in the subarachnoid space).[51] In dogs the pronounced renal vasoconstriction caused by cyclopropane was found to be neurogenic in origin,[58] and evidence was obtained that this is also true in man.[59] Hypertensive and sympathoadrenal responses to cyclopropane in dogs were found to disappear together as the brain was sectioned from above downward.† The lowest level of section at which the responses persisted was just above the pressor area of the medulla. Finally, preliminary evidence suggests that cardiac contractility is severely depressed by cyclopropane in man after blockade of cardiac sympathetic nerves.*

It should be noted that arterial hypotension, decreased stroke volume, and increased ventricular filling pressure often occur during the induction of anesthesia with cyclopropane. The reversal of these changes as time progresses suggests the operation of some homeostatic mechanism. Barostatic reflexes come to mind at once in this connection and are presumably important in the response. But we still have to explain why arterial pressure and stroke volume, instead of merely reattaining their normal levels, continue to increase until they are conspicuously greater than before the administration of cyclopropane. At the moment it appears that cyclopropane may diminish the sensitivity of the medullary vasomotor area to afferent inhibitory impulses, thus in effect setting the "barostat" at a higher than normal level.

What is the physiologic significance of these actions? Obviously this cannot be decided finally from the limited knowledge currently available, but certain inferences can be drawn. It appears that the maintenance of near normal levels of myocardial contractility during cyclopropane anesthesia depends upon the high level of sympathetic tone which the drug elicits. This may well be desirable, although there is evidence that cyclopropane "sensitizes" the myocardium to the arrhythmogenic actions of catecholamines[30] and that this action, together with the increased discharge of cardiac sympathetic nerves, may initiate ventricular extrasystoles and other cardiac arrhythmias.[71] Visceral vasoconstriction which occurs simultaneously is apparently also of sympathetic nervous origin; this is perhaps undesirable. It appears doubtful that the demand of renal or hepatic cells

*T. D. Darby: Personal communication.

†H. L. Price: Unpublished observations.

*H. L. Price: Unpublished observations.

for oxygen could be reduced by cyclopropane to the same extent that renal and hepatic blood flows have been reported to fall when the gas is inhaled. In other words, some degree of tissue ischemia may be present in these areas. In the case of the kidney, it is interesting that the lowest blood flows were found during the induction of anesthesia and tended to return partially to normal levels during a subsequent period of anesthesia and operation.[37] While there are a number of possible explanations for this finding, a not unlikely one is the accumulation of metabolites in the ischemic renal tissues.

Finally, there is a price to be paid for chronically heightened sympathetic nervous activity which takes, among others, the form of hypotension after withdrawal of the sympathetic stimulus.[14, 25] The question naturally arises about what part this effect plays in explaining arterial hypotension which sometimes follows termination of cyclopropane anesthesia.[24] Although this cannot be definitely answered at present, it can be said that no facts are known which would deny such an explanation.

### Ether

In a number of ways the actions of ether are similar to those of cyclopropane. Despite the ability of ether to depress myocardial contractility in the heart-lung preparation,[68] contractile force is only moderately reduced in intact animals.[7] Again, the difference between responses in intact and isolated preparations is believed attributable to increased sympathetic nervous discharge in the intact organism.[8, 70, 72] Cardiac output is increased remarkably during light planes of anesthesia, both in dogs[2, 5, 6, 8, 80] and men.[46, 65, 77] Tachycardia is also a conspicious feature of the response; it depends apparently both upon increased sympathetic nervous activity and upon vagal blockade.[27] Vasoconstriction occurs in the kidney[18, 37] and spleen,[38] and there is evidence that this vasoconstriction is neurogenic in origin.[3, 22, 59]

But after this point differences between the hemodynamic responses to ether and cyclopropane become increasingly evident. Total peripheral resistance is diminished even during profound ether narcosis,[6, 46] and arterial pressure therefore declines progressively if cardiac output is reduced by deepening anesthesia. Vasoconstriction in the viscera is not so pronounced as during cyclopropane anesthesia; indeed nearly complete blood vessel atonia and circulatory stasis have been described in certain tissues.[41]

It seems implausible to argue that such a response represents merely a reflex barostatic response to the direct cardiovascular depressant actions of ether. If it were, increased total peripheral resistance would certainly accompany arterial hypotension. Instead, arterial hypotension coexists with reduced peripheral resistance, which leads one to suspect that barostatic reflexes are not functioning normally. In dogs, reflex vasomotor responses to changes in carotid sinus pressure were virtually absent during profound ether anesthesia,[2, 12, 94] and clinical studies suggest a similar effect in man.[84] But, while the carotid occlusion test for baroreceptor activity failed almost completely in dogs under ether anesthesia, some baroreceptor activity must have been present because destruction of the sinus nerves led to pronounced arterial hypertension.[12] Barring the unlikely possibility that carotid occlusion failed to reduce either pressure or pulsations in the sinus, this means that ether in some way rendered the carotid sinus mechanism relatively insensitive to pressure reduction without abolishing its ability to reduce systemic arterial pressure. In other words, ether "sensitized" the carotid sinus mechanism. A limited amount of direct evidence for this action has been found in cats anesthetized with chloralose.[82, 83] Unfortunately, the periods of exposure to ether were brief and the anesthetic concentrations high in these experiments; but, supposing that this action occurs at lower concentrations, in all species, and irrespective of the duration of anesthesia, it could explain the coexistence

of reduced peripheral resistance and arterial hypotension.

It could not explain the occurrence of tachycardia, increased cardiac output, or increased sympathetic nervous activity. Derouaux reported in 1909 that in normal dogs, intracarotid injection of 3 ml. of saline solution saturated with ether caused tachycardia, arterial hypertension, and an increase in the force of cardiac contraction.[22] Injections of saline alone had no effect. From other studies this author concluded that ether acted in the central nervous system (probably in the medulla oblongata) in some manner which provoked increased sympathetic nervous activity. Since this remarkable essay, little of real importance concerning the means by which ether induces such activity has found its way into print. It may be significant that concentrations of ether which paralyze pressoreceptors can stimulate chemoreceptor activity[48, 50] because stimulation of chemoreceptors could explain not only the sympathoadrenal responses but also the hyperventilation which characteristically accompanies ether inhalation. It is suggestive that Derouaux[22] found the intracarotid injection of ether to be fcllowed by marked hyperventilation.

Peripheral actions of ether appear as poorly defined as those exerted in the central nervous system. The responsiveness to epinephrine of terminal arterioles and precapillaries in the canine omentum was reduced and vasomotion was completely suppressed by "deep" ether anesthesia; vasodilatation was marked and blood flow sluggish.[41] Responses to norepinephrine were not studied. Again in the dog, ether converted the pressor response to a depressor one ("epinephrine reversal"),[2] while in man the pressor response to intravenous infusions of norepinephrine was halved by the administration of ether.[72] Clearly the evidence is not all in, but it is at least conceivable that total peripheral resistance is reduced during ether anesthesia because the anesthetic interferes with the ability of blood vessels to constrict in response to increased sympathetic nervous activity. Other possibilities include direct (dilator) action on blood vessels,[58] reduced blood viscosity,[22] and metabolic acidosis.[15, 70]

These findings are, to be charitable, inconclusive. Two statements which can be made at the moment are that ether acts in the central nervous system in some manner which provokes a rather general increase in sympathetic nervous activity and that this is a fortunate thing (if one favors the use of ether), because otherwise administration of the anesthetic would be attended by cardiac incompetence, profound arterial hypotension, and perhaps death. Discussion of the significance of this state of affairs is analogous to that already detailed in considering actions of cyclopropane with two important differences. First the administration of ether does not cause such intense visceral vasoconstriction as cyclopropane.[18, 37] Second, ether appears not to "sensitize" the heart to the arrhythmogenic actions of catecholamines.[63]

### Halothane ($CF_3$ CH ClBr)

Several hemodynamic effects of halothane represent antitheses of those caused by cyclopropane. In man cardiac contractile force (strain gage arch) was poorly maintained during halothane inhalation; levels as low as 20 per cent of normal were recorded.[91] Stroke volume[57, 87] and cardiac output[57, 87, 101] diminished despite increased central venous pressure.[87] Heart rate[57, 101] and arterial pressure[57, 87, 101] were also reduced, but total systemic peripheral resistance was only slightly and inconsistently affected.[57, 87, 101] Arterial hypotension thus was primarily attributable to reduced cardiac output. Atropine was effective in reversing bradycardia but less successful in correcting arterial hypotension and reduced cardiac output.[57, 87, 101] Blood flow and vascular resistance in the viscera have not been reported, but vascular resistance in perfused hind limbs of dogs was reduced when halothane was inspired by a donor animal.[17] If this action of halothane occurs generally in the body, arterial hypo-

tension need not imply inadequate tissue perfusion.

Initially it was suggested that splanchnic vasodilatation, caused by sympathetic ganglion blockade, was the main cause of arterial hypotension during the administration of halothane.[79] However, it was later shown that halothane caused hypotension even in eviscerated animals and that its ability to block ganglionic transmission actually was rather limited.[16] Results in man also appear to rule out ganglionic blockade as the cause of hypotension.[87]

A more likely site of action is in the central nervous system, where halothane may act to suppress sympathetic nervous activity and possibly to increase cardiac vagal activity. Although a central action has not yet been shown directly, its existence is a logical inference from the evidence available. It has been shown in dogs that the pressor response to carotid occlusion is virtually abolished when anesthetic concentrations of halothane are respired.[79] Moreover, the failure of this reflex appears not to result from actions on baroreceptors,[99] on ganglionic transmission,[16] or on effector cells.[79] This by exclusion suggests an action in the central nervous system. Findings in man are consistent with this explanation, for despite the low level of arterial pressure which often obtains, analyses of plasma for catecholamines have not revealed evidence of increased sympathetic nervous activity.[72] In addition, evidence for suppression of central sympathetic representations by halothane has been secured by estimating the sympathoadrenal response to carbon dioxide inhalation in man.[76] These findings should not be taken to mean that sympathetic nervous activity fails to increase at all during halothane anesthesia, but there is remarkably little change considering the low levels of arterial pressure which may occur.

Turning to peripheral actions, effects diametrically opposite to those of cyclopropane are again encountered. Halothane, besides relaxing artificially perfused blood vessels in situ,[17] reduced the response of vascular smooth muscle to the sympathetic mediator, norepinephrine.* Interestingly, both findings may be different manifestations of the same action, that is, reduced effectiveness of the sympathetic mediator in the presence of halothane. Halothane also reduced cardiac responses to catecholamines.[61] Hence, to the fact that sympathetic nervous activity is relatively weak during halothane anesthesia must be added the observation that whatever activity does exist will probably be of reduced effectiveness because of peripheral actions of the anesthetic. This similarity between central and peripheral effects of halothane suggests again that a single mode of action (reduced effectiveness of norepinephrine) may be involved in all areas. It is known that central nervous sympathetic representations are rich in norepinephrine.[96] Moreover, norepinephrine has been suggested as a central nervous autonomic mediator.[9] Since it is possible to view the development of nerves as the answer of a large animal to the need for release of chemical mediators at distant sites, it is also reasonable to expect similarities between central and peripheral actions of the mediators as well as similarities between the central and peripheral effects of drugs which modify these actions.

What is the functional significance of the hemodynamic effects of halothane? At present this is uncertain. It is certain that tissue blood flow cannot be estimated by measuring arterial pressure. Undoubtedly hypotension can cause tissue ischemia, but ordinarily one cannot conclude that ischemia exists merely because hypotension is present. Of course, the fact that cardiac output is reduced by halothane means that some tissues receive less than their normal blood flow. But is reduced flow imposed on the tissues because of primary failure of the heart as a pump or is it a simple consequence of reduced metabolic demand caused by the anesthetic? One method of deciding consists of comparing whole body

*M. L. Price: Unpublished observations.

oxygen consumption with cardiac output before and during halothane administration. This has been done,[87] but not in a readily interpretable manner, and so the question remains open. On the other hand, if one accepts the evidence from direct measurements of cardiac contractile force, a point will be reached as halothane anesthesia is deepened at which the heart fails mechanically. Apparently, this is a weak point of the response, and it appears that this weakness results from perversion of autonomic regulation in company with direct myocardial actions of halothane.

### Thiopental

The hemodynamic effects of thiopental are distinct from those of any of the anesthetics so far discussed. Characteristically, cardiac output was moderately (15 to 20 per cent) reduced, while heart rate and total peripheral resistance increased by a similar amount during "deep" anesthesia in man.[28] Arterial pressure was inconsistently affected, although it was usually slightly reduced. The addition of nitrous oxide (50 to 70 per cent by inhalation) did not appear to modify the response unduly, although arterial pressure was less well maintained and peripheral resistance failed to increase as consistently as in the study referred to above.[26] Essentially similar results were obtained in another study whose subjects were patients undergoing surgical operations while anesthetized with thiopental and nitrous oxide.[31] Central venous pressure decreased slightly or remained unchanged,[26, 31] while central blood volume diminished to the same extent as did cardiac output.[28] several authors noted that cardiac output diminished progressively with increasing depth of anesthesia and that the decrease in output was largely attributable to reduced stroke volume.[28, 31]

It needs emphasis that hemorrhage and intermittent positive pressure lung ventilation produce circulatory changes similar to those caused by thiopental; therefore, results obtained during surgical operations or controlled respiration must be evaluated cautiously. In this connection it is worth noting that an opiate was administered before the induction of anesthesia in all of the studies cited above; whether or not opiates modify the circulatory response to thiopental (as they do with cyclopropane) is unknown.

Vascular resistance in the viscera apparently changes relatively slightly in response to thiopental injection. Splanchnic vascular resistance was found to be unaltered in one clinical study,* while renal vascular resistance increased by roughly 25 per cent in another.[37] Resistance in the extremities was transiently reduced.[52, 78] Cerebral vascular and myocardial vascular resistance were usually slightly to moderately elevated.[33, 43, 65, 86, 89]

In brief, the salient hemodynamic feature of thiopental anesthesia is that arterial pressure is well maintained while cardiac output and tissue blood flow are reduced. This implies increased vascular resistance. The important questions for our purpose are how and why these changes occur.

Against the possibility that these findings result primarily from myocardial depression stand a number of strong arguments. First, filling pressure is not elevated.[26, 31] Second, direct measurements of cardiac contractile force have revealed relatively little depression.[19, 60, 68] Third, there is no evidence for a reflexly mediated increase in sympathetic nervous activity[56, 72] such as would be expected to occur if the heart failed to provide an adequate output. This assumes that adequate reflex activity is possible during thiopental anesthesia; apparently it is during "light" anesthesia, although deeper planes tend to suppress it.[66]

A more likely explanation for reduced cardiac output is that it stems from reduced metabolic demand. Evidence for this follows. Whole body oxygen consumption during thiopental anesthesia was found to be diminished by about one-fifth compared with predicted basal values,[23] i.e., by roughly the same amount as the reduc-

*R. M. Epstein: Personal communication.

tion in cardiac output found in other studies. A similar relation exists in the case of the cerebral circulation[43, 85, 98] and probably also in that of the myocardium.[65] In both areas, vascular resistance is nearly independent of innervation and depends basically on the intrinsic ability of blood vessels to adjust flow to metabolic demand. All tissues apparently possess this ability, although it can be overshadowed by sympathetic nervous activity in heavily innervated regions. When sympathetic nervous activity is minimal, as it apparently is during thiopental administration, it is reasonable to expect vascular resistance to increase pari passu with any diminution in metabolic demand.

This argument is valid only provided thiopental does not interfere with the intrinsic reactivity of blood vessels. There is some evidence that thiopental causes vasoconstriction by acting directly on blood vessels,[35] but whether this occurs in the concentration range encountered clinically is unknown, and it seems somewhat improbable. Observations of the omental circulation in dogs suggest good maintenance of vascular reactivity until profound levels of anesthesia are attained.[41]

On the basis of this analysis, then, thiopental receives a relatively clean "bill of health." However, from a practical standpoint, this appraisal is of somewhat limited value because the anesthetic actions of the drug are so feeble that in practice it is almost always supplemented by employing other drugs—particularly nitrous oxide, opiates, and muscle relaxants—which themselves may have important circulatory actions. In addition it is usually given intravenously, and relatively large amounts of thiopental can accumulate briefly in the viscera, including the heart, after its intravenous injection.[75] The effects of these transient "overdoses" have not been adequately studied.

## Conclusion

The conjectural nature of the foregoing needs no emphasis. This arises from the necessity of assigning significance to observations which are themselves superficial in character. Deeper understanding awaits better methods and better measurements. Yet certain outlines are visible. These can best be illustrated by comparing halothane and cyclopropane.

The fact that human beings can survive a reduction in myocardial contractile force as great as 80 per cent (reported during halothane anesthesia) indicates an ability of the circulatory apparatus to function adequately under highly unfavorable circumstances. This adaptability, shared apparently both by blood vessels and heart, makes it nearly impossible to insist that certain levels of arterial pressure or even of tissue blood flow are "critical" for the preservation of function. What we need to learn, by direct measurement if possible, is how satisfactorily the metabolic needs of the tissues are being served by the circulation. Obviously, the methods necessary to answer this may turn out not to be "circulatory" at all.

At present, it appears wisest to view any marked departures from normal levels of function as suspect. For example, the fact that cardiac output during halothane administration may be maintained at levels which approach normal, even though contractile force is greatly diminished, is itself intriguing; this has been discussed at length elsewhere.[74] But undoubtedly halothane would be a safer drug if it did not depress contractility so markedly, and the mechanism responsible for this effect is therefore of primary importance. Seemingly it results not from an outstanding ability of halothane to depress myocardial function directly, but from sympathetic paresis combined with cardiac actions which are a common property of all general anesthetic agents. Is such an action on the sympathetic nervous system desirable in itself? It is hard to make a case for it, although there have been attempts to do so.[44] The difficulty stems from having to argue that weakening a powerful compensatory mechanism can somehow increase

the chance of survival under unfavorable conditions.

Several responses to cyclopropane appear at the opposite pole from those evoked by halothane. Again it may be asked whether there is anything intrinsically desirable about these actions. The answer to this also appears negative. But it would be strongly affirmative if the word "intrinsic" were omitted from the question, for, to the extent that these reactions can preserve adequate myocardial contractility in the face of anesthetic depression of the heart, they are undeniably vital.

A similar comparison can be made in considering tissue circulation. It would appear that tissue survival is most likely in the presence of drugs which interface minimally with the responses of the fine circulation to their normal stimuli (including catecholamines). Again, the theoretic argument seems to favor cyclopropane, and there is also some practical experience in support of it.[20, 39, 40, 102] Vasoconstriction, if sufficiently intense and prolonged, could be damaging and this possibility has been considered in discussing the fact that cyclopropane undoubtedly can cause vasoconstriction in a number of vascular areas. However, there is no positive evidence that damage does occur, and unless it can be shown that cyclopropane actively prevents precapillary sphincters from dilating even when metabolites accumulate nearby, it appears reasonable to prefer the degree of vasoconstriction which it produces to atonia and unresponsiveness of the finer blood vessels.

### *References*

1. Acierno, L. J., and DiPalma, J. R.: Effects of ether, cyclopropane and chloroform on the isolated auricle of the cat, Anesthesiology **12**: 567-573, 1951.
2. Albers, C., Brendel, W., and Usinger, W.: Kreislauf respiratorischer Stoffwechsel und Atmung in Athernarkose, Arch. exper. Path. u. Pharmakol. **226**:278-300, 1955.
3. Bhatia, B. B., and Burn, J. H.: The action of ether on the sympathetic system, J. Physiol. **78**:257-270, 1933.
4. Blacket, R. B., Pickering, G. W., and Wilson, G. M.: Effects of prolonged infusions of noradrenaline and adrenaline on the arterial pressure of the rabbit, Clin. Sc. **9**:247-256, 1950.
5. Blalock, A.: The effect of ether anesthesia on cardiac output, Arch. Surg. **14**:732-751, 1927.
6. Blalock, A.: The effects of ether, chloroform and ethyl chloride anaesthetics on the minute cardiac output and blood pressure, Surg. Gynec. & Obst. **46**:72-78, 1928.
7. Boniface, K., Brown, J., and Kronen, P.: The influence of some inhalation anesthetic agents on the contractile force of the heart, J. Pharmacol. & Exper. Therap. **113**:64-71, 1955.
8. Brewster, W. R., Jr., Isaacs, J. P., and Wainø-Andersen, T.: Depressant effect of ether on myocardium of the dog and its modification by reflex release of epinephrine and norepinephrine, Am. J. Physiol. **175**:399-414, 1953.
9. Brodie, B. B., and Shore, P. A.: A concept for a role of serotonin and norepinephrine as chemical mediators in the brain, Ann. New York Acad. Sc. **66**:631, 1957.
10. Brown, G. L., and Gillespie, J. S.: Output of sympathin from the spleen, Nature, London **178**:980, 1956.
11. Brown, G. L., and Gillespie, J. S.: The output of sympathetic transmitter from the spleen of the cat, J. Physiol. **138**:81-102, 1957.
12. Brown, R. V., and Hilton, J. G.: The effectiveness of the baroceptor reflexes under different anesthetics, J. Pharmacol. & Exper. Therap. **118**:198-203, 1956.
13. Buckley, N. M., Ogden, E., and McPherson, R. C.: The effect of inotropic drugs on filling of the isolated right ventricle of the dog heart, Circulation Res. **3**:447-453, 1955.
14. Bülbring, E., and Burn, J. H.: An action of adrenaline in sympathetic synapses which may play a part in shock, J. Physiol. **101**:289-296, 1942.
15. Bunker, J. P., Beecher, H. K., Briggs, B. D., Brewster, W. R., and Barnes, B. A.: Metabolic effects of anesthesia. II. A comparison of the acid-base equilibrium in man and dogs during ether and cyclopropane anesthesia, J. Pharmacol. & Exper. Therap. **102**:62-70, 1951.
16. (A) Burn, J. H., Epstein, H. G., Feigan, G. A., and Paton, W. D. M.; (B) Burns, T. H. S., Mushin, W. H., Organe, G. S. W., and Robertson, J. D.: "Fluothane": A report to the medical research council by the committee on non-explosive anesthetic agents, Brit. M. J. **2**:479-490, 1957.
17. Burn, J. H., and Epstein, H. G.: Hypotension due to halothane, Brit. J. Anaesth. **31**:199-204, 1959.

18. Burnett, C. H., Bloomberg, E. L., Shortz, G., Compton, D. W., and Beecher, H. K.: A comparison of the effects of ether and cyclopropane anesthesia on the renal function of man, J. Pharmacol. & Exper. Therap. **96**:380-387, 1949.
19. Cotten, M. D., and Bay, E.: Comparison of the cardiovascular properties of a new nonbarbiturate intravenous anesthetic agent with those of thiopental, Anesthesiology **17**:103-111, 1956.
20. Crooke, A. C., Morris, C. J. O. R., and Bowler, R. G.: General anesthesia in shock, Brit, M. J. **2**:683-689, 1944.
21. Daniel, E. E., Fulton, J. B., Hiddleston, M., Martin, W., and Foulks, J. G.: An analysis of the mechanism of barbiturate induced cardiovascular depression and its antagonism by sympathomimetic amines, Arch. internat. pharmacodyn. **108**:457-472, 1956.
22. Derouaux, J.: Nouvelles recherches sur l'action physiologique de l'éther sulfurique, Arch internat. pharmacodyn. **19**:63-95, 1909.
23. Dobkin, A. B., and Wyant, G. M.: The physiological effects of intravenous anesthesia on man, Canad. Anaesth. Soc. J. **4**:295-337, 1957.
24. Dripps, R. D.: The immediate decrease in blood pressure seen at the conclusion of cyclopropane anesthesia. "Cyclopropane shock," Anesthesiology **8**:15-35, 1947.
25. Duner, H., and von Euler, U. S.: Secondary fall in blood pressure following noradrenaline infusion in the cat, Acta physiol. scandinav. **38**:355-363, 1956.
26. Elder, J., Nagano, S., Eastwood, D., and Harnagel, D.: Circulatory changes associated with thiopental anesthesia in man, Anesthesiology **16**:394-400, 1955.
27. Elfstrand, M.: Boebachtungen über die Wirkung einger aliphatischer Kohlenwasserstoffe, verglichen mit derjenigen des Aethers, und über das Verhalten der Vaguserregbarkeit während der Narkose, Arch. exper. Path. u. Pharmakol **43**: 435-455, 1899-1900.
28. Etsten, B., and Li, T. H.: Hemodynamic changes during thiopental anesthesia in humans: Cardiac output, stroke volume, total peripheral resistance and intrathoracic blood volume, J. Clin. Invest. **34**:500-510, 1955.
29. Evans, C. L., Tsai, C., and Young, F. G.: The behavior of liver glycogen in experimental animals. I. Methods: The effect of ether and amytal, J. Physiol. **73**: 67-80, 1931.
30. Fawaz, G.: Mechanism by which N:N-dibenzylchloroethylamine protects animals against cardiac arrhythmias produced by sympathomimetic amines in presence of cyclopropane or chloroform, Brit. J. Pharmacol. **6**:492-500, 1951.
31. Fieldman, E., Ridley, R., and Wood, E.: Hemodynamic studies during thiopental sodium and nitrous oxide anesthesia in humans, Anesthesiology **16**:473-489, 1955.
32. Fisher, C. W., Bennett, L. L., and Allalwala, A.: Effect of inhalation anesthetic agents on the myocardium of the dog, Anesthesiology **12**:19-26, 1951.
33. Geiger, A., and Magnes, J.: The isolation of the cerebral circulation and the perfusion of the brain in the living cat, Am. J. Physiol. **149**:517-537, 1947.
34. Gruber, C. M.: Effects of anesthetic doses of Na-thiopentobarbital, Na-thio-ethamyl and pentothal Na upon the respiratory system the heart and blood pressure in experimental animals, J. Pharmacol. & Exper. Therap. **60**: 143-173, 1937.
35. Gruber, C. M., Gruber, C. M., Jr., and Lee, K. S.: A study of the effect of thiobarbiturates on the cardio-vascular system, Arch. internat. pharmacodyn. **91**:461-488, 1952.
36. Gruhzit, C. C., and Farah, A. E.: A comparison of the positive inotropic effects of ouabain and epinephrine in heart failure induced in the dog heart-lung preparation by sodium pentobarbital, dinitrophenol, sodium cyanide and sodium azide, J. Pharmacol. & Exper. Therap. **114**:334-342, 1955.
37. Habif, D. V., Papper, E. M., Fitzpatrick, H. F., Lowrance, P., Smythe, C. M., and Bradley, S. E.: The renal and hepatic blood flow, glomerular filtration rate and urinary output of electrolytes during cyclopropane, ether and thiopental anesthesia, operation and the immediate postoperative period, Surgery **30**: 241-255, 1951.
38. Hausner, E., Essex, H., and Mann, F. C.: Roentgenologic observations of the spleen of the dog under ether, sodium amytal, pentobarbital sodium, and pentothal sodium anesthesia, Am. J. Physiol. **121**:387-391, 1938.
39. Hershey, S. G., Zweifach, B. W., Chambers, R., and Rovenstine, E. A.: Peripheral circulatory reactions as a basis for evaluating anesthetic agents, Anesthesiology **6**:362-375, 1945.
40. Hershey, S. G., and Zweifach, B. W.: Peripheral vascular homeostasis in relation to anesthetic agents, Anesthesiology **11**:145-154, 1950.
41. Hershey, S. G., Zweifach, B. W., and Rovenstine, E. A.: Effects of depth of anesthesia on behavior of peripheral vascular bed, Anesthesiology **14**:245-254, 1953.
42. Heymans, C., and Neil, E.: Reflexogenic areas of the cardiovascular system, Boston, 1958, Little, Brown & Company, p. 102.

43. Himwich, W. A., Homburger, E., Maresca, R., and Himwich, H. E.: Brain metabolism in man: Unanesthetized and in pentothal narcosis, Am. J. Psychiat. **103**:689-696, 1946-1947.
44. Johnstone, M.: The role of halothane in the prevention of surgical shock, Brit. J. Anaesth. **30**:435-448, 1958.
45. Jones, R. E., Guldmann, N., Linde, H. W., Dripps, R. D., and Price, H. L.: Cyclopropane anesthesia. III. Effects of cyclopropane on respiration and circulation in normal man, Anesthesiology **21**:380-393, 1960.
46. Jones, R. E., Deutsch, S., Linde, H. W., Dripps, R. D., and Price, H. L.: Hemodynamic actions of diethyl ether in normal man. To be published.
47. Kitchin, A. H., Sanger, C., DeWardener, H. E., and Young, M. I.: The blood flow in human muscle during cyclopropane anaesthesia, Clin. Sc. **12**:361-366, 1953.
48. Landgren, S., Liljestrand, G., and Zotterman, Y.: Wirkung von Alcohol, Aceton, Äther und Chloroform auf die Chemoceptoren des Glomus caroticum, Arch. exper. Path. u. Pharmakol. **219**:185-191, 1953.
49. Li, T. H., and Etsten, B.: Effect of cyclopropane anesthesia on cardiac output and related hemodynamics in man, Anesthesiology **18**:15-32, 1957.
50. Liljestrand, G.: The effects of ethyl alcohol and some related substances on baroreceptor and chemoceptor activity, Acta physiol. scandinav. **29**:74-82, 1953.
51. Lurie, A. A., Jones, R. E., Linde, H. W., Price, M. L., Dripps, R. D., and Price, H. L.: Cyclopropane Anesthesia. I. Cardiac rate and rhythm during steady levels of cyclopropane anesthesia at normal and elevated end-expiratory carbon dioxide tensions, Anesthesiology **19**:457-472, 1958.
52. Lynn, R. B., and Shackman, R.: The peripheral circulation during general anaesthesia and surgery, Brit. M. J. **2**:333-336, 1951.
53. Mann, M., and West, G. B.: The nature of hepatic and splenic sympathin, Brit. J. Pharmacol. **5**:173-177, 1950.
54. Mann, M., and West, G. B.: Nature of uterine and intestinal sympathin, Brit. J. Pharmacol. **6**:79-83, 1951.
55. Manning, J. W., Jr., and Peiss, C. N.: Cardiovascular responses elicited by electrical stimulation of the hypothalmus, Fed. Proc. **17**:104, 1958.
56. McAllister, F. F.: The effect of pentothal sodium on mean arterial blood pressure in the presence of high spinal cord paralysis, Ann. Surg. **124**:328-331, 1946.
57. McGregor, M., Davenport, H. T., Jegier, W., Sekely, P., Gibbons, J. E., and Demers, P. P.: The cardiovascular effects of halothane in normal children, Brit. J. Anaesth. **30**:398-408, 1958.
58. Miles, B. E., and DeWardener, H. E.: Renal vasoconstriction produced by ether and cyclopropane anaesthesia, J. Physiol. **118**:140-144, 1952.
59. Miles, B. E., DeWardener, H. E., Churchill-Davidson, H. C., and Wylie, W. D.: The effect on the renal circulation of pentamethonium bromide during anesthesia, Clin. Sc. **11**:74-79, 1952.
60. Moe, G. K., Rennick, B. R., Freyburger, W. A., and Malton, S. D.: The effect of cyclopropane on cardiac work capacity, Anesthesiology **10**:706-713, 1949.
61. Nayler, W. G.: The action of fluothane, chloroform and hypothermia on the heart, Austral. J. Exper. Biol. & M. Sc. **37**:279-288, 1959.
62. Nowak, S. J. G.: The effect of evipan, pernocton, sodium amytal and avertin upon circulation and respiration with reference to their effect on the carotid sinus reflex, Arch. internat. pharmacodyn. **60-61**:118-128, 1938-39.
63. Orth, O. S., Leigh, M. D., Mellish, C. H., and Stutzman, J. W.: Action of sympathomimetic amines in cyclopropane, diethyl ether, and chloroform anesthesia, J. Pharmacol. & Exper. Therap. **67**:1-16, 1939.
64. Outschoorn, A. S., and Vogt, M.: The nature of cardiac sympathin in the dog, Brit. J. Pharmacol. **7**:319-324, 1952.
65. Pellegrini, G.: Der Einfluss der Barbiturnarkose auf die Koronardurchbluting, Verhandl. deutsch. Gesellsch. Kreis. **23**:111-112, 1957.
66. Price, H. L., Conner, E. H., Elder, J. D., and Dripps, R. D.: Effect of sodium thiopental on circulatory response to positive pressure inflation of lung, J. Appl. Physiol. **4**:629-635, 1952.
67. Price, H. L., Conner, E. H., and Dripps, R. D.: Concerning the increase in central venous and arterial blood pressure during cyclopropane anesthesia in man, Anesthesiology **14**: 1-9, 1953.
68. Price, H. L., and Helrich, M.: The effect of cyclopropane, diethyl ether, nitrous oxide, thiopental and hydrogen ion concentration on the myocardial function of the dog heart-lung preparation, J. Pharmacol. & Exper. Therap. **115**:206-216, 1955.
69. Price, H. L., and Price, M. L.: The chemical estimation of epinephrine and norepinephrine in human and canine plasma. II. A critique of the trihydroxyindole method, J. Lab. & Clin. Med. **50**:769-777, 1957.
70. Price, H. L.: Circulating adrenaline and

noradrenaline during diethyl ether anaesthesia in man, Clin. Sc. **16**:377-387, 1957.

71. Price, H. L., Lurie, A. A., Jones, R. E., Price, M. L., and Linde, H. W.: Cyclopropane anesthesia. II. Epinephrine and norepinephrine in initiation of ventricular arrhythmias by carbon dioxide inhalation, Anesthesiology **19**:619-630, 1958.
72. Price, H. L., Linde, H. W., Jones, R. E., Black, G. W., and Price, M. L.: Sympathoadrenal responses to general anesthesia in man and their relation to hemodynamics, Anesthesiology **20**:563-575, 1959.
73. Price, H. L., Linde, H. W., and Price, M. L.: Failure of ethylenediamine condensation method to detect increased plasma norepinephrine concentrations during general anesthesia in man, CLIN. PHARMACOL. & THERAP. **1**:298-302, 1960.
74. Price, H. L.: General anesthesia and circulatory homeostasis, Physiol. Rev. **40**:187-218, 1960.
75. Price, H. L., Kovnat, P. J., Safer, J. N., and Price, M. L.: The uptake of thiopental by body tissues and its relation to the duration of narcosis, CLIN. PHARMACOL. & THERAP. **1**:16-22, 1960.
76. Price, H. L.: Modification by general anesthetics (cyclopropane and halothane) of circulatory and sympathoadrenal responses to respiratory acidosis, Ann. Surg., 1960. In press.
77. Prime, F. J., and Gray, T. C.: The effect of certain anaesthetic and relaxant agents on circulatory dynamics, Brit. J. Anaesth. **24**:101-136, 1952.
78. Prime, F. J., and Gray, T. C.: The effect of certain anaesthetic and relaxant agents on circulatory dynamics, Brit. J. Anaesth. **24**: 101, 1952.
79. Raventos, J.: The action of fluothane—a new volatile anaesthetic, Brit. J. Pharmacol. **11**: 394-410, 1956.
80. Remington, J. W., Hamilton, W. F., Wheeler, N. C., and Hamilton, W. F., Jr.: Validity of pulse contour for calculating cardiac output of the dog, with notes on effect of various anesthetics, Am. J. Physiol. **59**:379-384, 1949.
81. Robbins, B. H., and Baxter, J. H., Jr.: Studies of cyclopropane. IV. Cardiac output in dogs under cyclopropane anesthesia, J. Pharmacol. & Exper. Therap. **62**:179-188, 1938.
82. Robertson, J. D., Swan, A. A. B., and Whitteridge, D.: Effect of anaesthetics on systemic baroreceptors, J. Physiol. **131**:463-472, 1956.
83. Robertson, J. D., and Swan, A. A. B.: Effect of anaesthetics on baroreceptor reflexes, Quart. J. Exper. Physiol. **42**:113-125, 1957.
84. Rovenstine, E. A., and Cullen, S. C.: The anesthetic management of patients with a hyperactive carotid sinus reflex, Surgery **6**: 167-176, 1939.
85. Schieve, J. F., and Wilson, W. P.: The influence of age, anesthesia and cerebral arteriosclerosis on cerebral vascular activity to $CO_2$, Am. J. Med. **15**:171-174, 1953.
86. Serra, P., and Urse, L.: Osservasioni reografiche sulla circolazione cerebrale in corso di narcosi, Minerva anestesiol. **22**:86-90, 1956.
87. Severinghaus, J. W., and Cullen, S. C.: Depression of myocardium and body oxygen consumption with fluothane, Anesthesiology **19**:165-177, 1958.
88. Shackman, R., Graber, I. G., and Melrose, D. G.: Liver blood flow and general anesthesia, Clin. Sc. **12**:307-315, 1953.
89. Sokoloff, L.: Factors regulating the total and regional circulation of the brain, Proceedings of the Third Micro-circulatory Conference, Milwaukee, 1956, pp. 79-88.
90. Thompson, M. C., Patrick, R. T., and Wood, E. H.: Effects of cyclopropane anesthesia on the circulation of human beings, J. A. M. A. **164**:389-393, 1957.
91. Thrower, W. B., Darby, T. D., Aldinger, E. E., and Sprouse, J. H.: Effects of halothane (fluothane) anesthesia on ventricular contractile force in the human and dog, Fed. Proc. **19**:274, 1960.
92. Tunnicliffe, F. W., and Rosenheim, O.: On the action of chloroform, ether, alcohol and acetone upon the excised mammalian heart, J. Physiol. **29**:15-16, 1903.
93. Valk, A. D., and Price, H. L.: The chemical estimation of epinephrine and norepinephrine in human and canine plasma. I. A critique of the ethylenediamine condensation method, J. Clin. Invest. **35**:837-841, 1956.
94. Vercauteren, E., and Heymans, C.: Influence de la chloralosane, de l'hyperventilation, du $CO_2$, de l'ésérine, du chloroforme, de l'éther, de la novacaïne, de la tutocaine, de la morphine, du somnifène de numal, et de la quinine sur les reflexes vasomoteurs du sinus carotidien, Compt. rend. soc. biol. Paris **109**: 563-565, 1932.
95. Vernon, H. M.: The mode of union of certain poisons with cardiac muscle, J. Physiol. **41**: 194-232, 1910-11.
96. Vogt, M.: The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs, J. Physiol. **123**:451-481, 1954.
97. von Euler, U. S., and Hellner, S.: Excretion of noradrenaline and adrenaline in muscular work, Acta physiol. scandinav. **26**:183-191, 1952.

98. Wechsler, R. L., Dripps, R. D., and Kety, S. S.: Blood flow and oxygen consumption of the human brain during anesthesia produced by thiopental, Anesthesiology **12**:308-314, 1951.
99. Whitteridge, D.: Effect of anaesthetics on mechanical receptors, Brit. M. Bull. **14**:5-7, 1958.
100. Wollenberger, A., and Krayer, O.: Experimental heart failure caused by central nervous system depressants and local anesthetics, J. Pharmacol. & Exper. Therap. **94**:439-443, 1948.
101. Wyant, G. M., Merriman, J. E., Kilduff, C. J., and Thomas, E. T.: The cardiovascular effects of halothane, Canad. Anaesth. Soc. J. **5**:384-402, 1958.
102. Zweifach, B. W., Hershey, S. G., Rovenstine, E. A., Lee, R. E., and Chambers, R.: Anesthetic agents as factors in circulatory reactions induced by hemorrhage, Surgery **18**: 48-65, 1945.

*In our general impressions far too great weight is attached to what is marvellous. . . . Experience warns us against it, and the scientific man takes care to base his conclusions upon actual numbers. The human mind is . . . a most imperfect apparatus for the elaboration of general ideas. . . . General impressions are never to be trusted. Unfortunately when they are of long standing they become fixed rules of life, and assume a prescriptive right not to be questioned. Consequently, those who are not accustomed to original enquiry entertain a hatred and a horror of statistics. They cannot endure the idea of submitting their sacred impressions to cold-blooded verification. But it is the triumph of scientific men to rise superior to such superstitions, to devise tests by which the value of beliefs may be ascertained, and to feel sufficiently masters of themselves to discard contemptuously whatever may be found untrue . . . the frequent incorrectness of notions derived from general impressions may be assumed. . . .*

FROM "GENERIC IMAGES"
BY F. GALTON, PROCEEDINGS
OF THE ROYAL INSTITUTE, 1879.

# Clinical pharmacology of digitalis materials

*The pharmacologic actions of digitalis which have been observed in the laboratory animal and in man are reviewed and correlated. It is shown how, in most instances, the findings in the former have a direct bearing on the application of digitalis materials in the clinic. An attempt is made to clarify some of the more poorly understood aspects of digitalis action.*

*Cardiac slowing by digitalis is explored and explained in some detail. The basis of digitalis dosage and cumulation is discussed. An attempt is made to indicate how knowledge of the pharmacologic actions of digitalis materials can be used to better exploit them therapeutically.*

**Walter Modell, M.D.*** *New York, N. Y.*
*Cornell University Medical College*

In textbooks of pharmacology, the actions of digitalis are usually neatly listed and then described in considerable detail. The drug has long been known to exert useful clinical effects on the heart, an organ physiologically and medically as well as symbolically very important. And, if not to interpret them, it has also long been known how to record many aspects of cardiodynamics. As a result, the effect of the digitalis glycosides on the heart has been the subject of an enormous amount of laboratory and clinical investigation and consequently of a voluminous literature. Because there is substantial understanding of both the pharamacologic actions and the clinical effects, satisfactory correlations can be drawn between the actions which can be demonstrated in the laboratory animal and the applications which can be put to the drug in the clinic. The reasons for some discrepancies between the two which seem to exist can also be acceptably explained. Thus the clinical pharmacology of these drugs has been well worked out and, therefore, the effective clinical use of these drugs can be well explained in terms of basic pharmacologic actions.

Fortunately, this is one area in pharmacology in which advertising and promotional claims for a plethora of synthetic congeners have not obfuscated the facts. Although there are a few semisynthetic derivatives, none are on the market. On the other hand, Nature has been most prolific, and an extremely large group of natural digitalis glycosides are available.[9] The chemical structure of the glycosides has been well worked out and is available in most pharmacology textbooks; it will not be considered here.[45, 46] Although claims for significant differences between them are often made, the best evidence, and the generally accepted view, is that all the digitalis glycosides exert the same basic pharmacologic actions, hence no significant

Based on a lecture presented at the University of Kansas Medical Center, Nov. 15, 1960.

Received for publication Nov. 15, 1960.

*Director, Clinical Pharmacology.

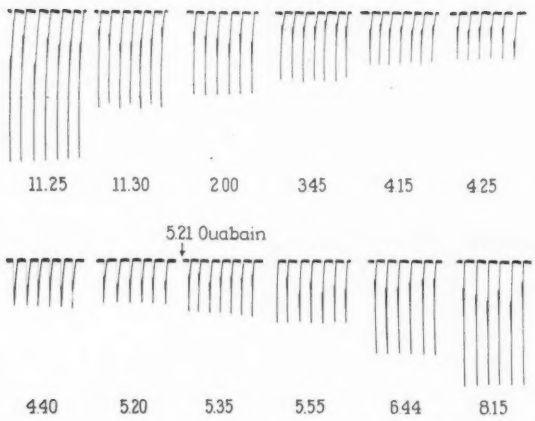



**Fig. 1.** The effect of ouabain on force of contraction of cardiac muscle. Note that the effect develops only after decay of normal contractile force. (From Cattell, M., and Gold, H.: J. Pharmacol. & Exper. Therap. 62:116, 1938, published by Williams & Wilkins Company.)

qualitative differences, and that the only important differences are in quantitative aspects of these actions.[39] In the discussion which follows, this view will be followed and digitalis will be used as the prototype of the whole group.

A standard textbook outline of digitalis actions usually considers those on (1) force of cardiac contraction, (2) sinus rate, (3) conduction, (4) cardiac output, (5) venous pressure, (6) arterial blood pressure, (7) coronary circulation, (8) excretion of urine, (9) cardiac automaticity, and the (10) central nervous system.

### Force of contraction

The effect of digitalis to increase the force of cardiac contraction may well be listed first, for this is the basis of its outstanding action in heart disease. Most of its important therapeutic effects can be related to this effect. The classic experiments of Cattell and Gold[7] on the isolated papillary muscle of the cat demonstrated the unique ability of digitalis to increase the force of systolic contraction. Perhaps because it was not shown in their illustration (Fig. 1), the point about this important experiment that is not so well remembered by those who use digitalis to exert this effect in man is that digitalis did not bring about supranormal contraction of the normal heart. No effect was demonstrable in these experiments when the muscle was *normal.* The critical feature of the experiment was that the muscle responded to digitalis only *after* and *not before* it had become *fatigued* (i.e., after relative failure had developed). Only then did use of digitalis restore force of contraction to normal.*

This corresponds with remarkable fidelity to the experience in the clinic. There is no evidence that digitalis increases the force of contraction in the normal heart or that it in any way exerts useful effects on the normal myocardium; thus it is of no use for the priming of marathon racers. The ionotropic (increase in contractile force) action of digitalis is of demonstrable clinical value only in heart failure. There is every reason to believe that the correlation between the experiences in the laboratory and in the clinic are based on the same unique action of digitalis to increase the force of contraction only of cardiac muscle which is weak, fatigued, or in some manner *failing.*[22]

### Sinus slowing

Digitalis can be shown to slow the heart in the dog. This does not occur in normal man. Even though the precise mechanism of the sinus slowing in the laboratory animal is not established, there is a satisfactory explanation for the apparent species differences.

Since it can be prevented with atropine, it seems evident that the moderate slowing demonstrable in the dog is somehow mediated through vagal mechanisms. Some postulate that it is the consequence of central stimulation, others offer both laboratory evidence and suggestive clinical experience to indicate that digitalis lowers the threshold of the sinoauricular node and

*A recent publication (J. Clin. Invest. 40:52, 1961) would seem to challenge this view, but on a quantitative basis it still stands. However, a study by Selzer and associates (Brit. Heart J. 21:335, 1959) indicates clearly that therapeutic doses of digoxin are without effect on the circulation of normal man.

thereby makes it more sensitive to the normal tonic vagal impulses. Finally, there are those who believe that there is a direct action on the heart which somehow slows it.[38] Regardless of which ultimately turns out to be the proper explanation, there is the fact that in the laboratory animal there is a degree of cardiac slowing by digitalis which is not seen in normal man. A species difference, which is very likely important, is that the normal heart rate in man (about 75) is relatively slow and under usual laboratory conditions that of the dog (about 150) is relatively high. In addition there is the fact, which may well be correlated with it, that the sinus rate in the dog is very responsive to vagal stimulation while it is not so responsive in normal man. This difference is seen in the response to morphine; in normal man the heart rate is little affected by small doses, whereas in the dog comparable doses of morphine may slow the heart sufficiently to permit ectopic ventricular beats to break through.

Despite the apparent difference, the vagal action of digitalis may nonetheless be identical in these species. Given a different circumstance, digitalis does induce and participate in sinus slowing in man. Thus, a large dose of digitalis will often bring about a cessation of a paroxysmal sinus tachycardia through intensification of vagal influence on the sinoauricular node. Other devices which sharply stimulate the vagal mechanism, an emetic, eyeball pressure, or carotid sinus pressure, may also terminate a paroxysm. In instances in which each type of device fails as such, a dose of digitalis, in some manner, apparently sensitizes the sinoauricular node to vagal influence so that the subsequent stimulation of the vagus by an emetic, eyeball pressure, or carotid sinus pressure may abruptly terminate a paroxysm which was uninfluenced by a similar maneuver previous to digitalis. Here, then, are instances in which digitalis can be shown to play a role in sinus slowing in man through a vagal mechanism. With a rapid sinus rate, the pacemaker in man seems to be more sensitive to this action of digitalis than when the rate is normal, and in this circumstance, when vagal tone is less influential, the effect of digitalis on the sinus rate in man tends to resemble that on the laboratory animal with a rapid rate.

There is another type of slowing by digitalis, unfortunately called "vagal" slowing, which may properly be clarified at this point. There is almost always some, at times considerable, sinus tachycardia in patients with heart failure. This is the consequence of a homeostatic reflex, an accelerator response arising from inefficient cardiac action. In such instances, the use of digitalis may be followed by slowing of the heart. In a very small part only is this likely to be caused by the mechanism discussed immediately above. By far the larger element of the slowing is likely to be the *indirect* consequence of the more important action of digitalis to increase the force of systolic contraction and thereby to provide relief from heart failure. In so doing, it simultaneously removes or reduces the functional stimulus to the accelerator mechanisms arising as a result of cardiac dysfunction; consequently, there is, in a manner of speaking, "reflex" slowing of the heart. That is to say, in reducing cardiac inefficiency through an ionotropic action, the stimulus to the accelerator mechanism is also reduced by the effects of digitalis and, as a result, the level of vagal tone is elevated toward normal. Although not really vagal in origin, since this reflex is mediated through vagal pathways it can be abolished with atropine. This explains why it is called "vagal" slowing even though it does not involve direct stimulation of any part of the vagal apparatus by digitalis.

It is so important that it seems to justify the reiteration that this is not a *primary* action on a vagal mechanism to bring about slowing but the *secondary* consequence of the action of digitalis on the myocardium to increase the force of systole and, thereby, to increase the efficiency of cardiac contraction. Even in heart failure, significant slowing of a rapid, but other-

wise normal, sinus mechanism cannot be induced by digitalis unless it first acts to relieve the heart failure by increasing the force of contraction. Thus in heart failure, even though the heart rate may be rapid, a primary slowing action of digitalis cannot be counted upon to significantly slow the *normal* pacemaker of the human heart.

### Conduction

Digitalis depresses conduction through the atrioventricular system (the bundle of His). In sufficiently large doses, it blocks conduction completely. In both the animal and man, this effect of digitalis on conduction can be demonstrated and followed in the electrocardiogram. In a normal sinus rhythm, after sufficiently large doses of digitalis, the depressant action on conduction produces progressive prolongation of the P-R interval, but there is still a one to one response of the ventricle to the sinus, and consequently there is *no slowing* of the heart by this action until second stage heart block develops, at which point, when there is, say, two to one block, the rate precipitously falls to about half the previous rate. Further digitalization induces complete atrioventricular dissociation with the extremely slow rates characteristic of the idioventricular pacemaker. That is, in the case of the normal pacemaker with all the P waves of about equal intensity, increasing conduction block does not prevent the transmission of any supraventricular impulses until it becomes intense enough to block about half. These effects of digitalis on conduction are, of course, major toxic effects. Hence conduction depression is not a useful action of digitalis in cases of heart failure with normal sinus rhythm. It may, however, be useful in patients with Adams-Stokes episodes, in whom it may be used to fix the heart block, thereby preventing the return to a normal ryhthm, hence ultimately preventing the opposite change in rhythm and the syncope associated with it.

The same action of digitalis on conduction, however, is of great clinical value in auricular fibrillation. As will be shown below, it makes possible the gradual slowing of the ventricular rate which digitalis so regularly brings about in cases of auricular fibrillation. But there is a special circumstance which makes this slowing action, the most dependable clinical effect of digitalis, possible in the case of auricular fibrillation.

In auricular fibrillation, the auricular impulses are irregular in intensity as well as in occurrence. The depressant action on conduction is able to slow the ventricular rate by suppressing the weaker auricular impulses while permitting the stronger ones to pass. As digitalis dosage is increased, to pass the digitalis barrier, the supraventricular stimulus must be increasingly stronger; consequently, since fewer and fewer of the impulses generated by the process of auricular fibrillation are potent enough to pass the barrier, the heart becomes progressively slower. Unless premature ventricular beats appear first, this continues as a gradual process until complete heart block develops and an idioventricular pacemaker assumes control of the ventricle. This gradual selective slowing is possible only because the auricular impulses are of unequal intensity, so that each increment in digitalization blocks out the weaker of the supraventricular beats which continue to stimulate the ventricle. The action is comparable to that of the grid which suppresses the passage of electrons in an electronic tube. This is in sharp contrast to the situation with a normal sinus rhythm, in which all auricular impulses are of equal intensity, so that a depressant action on conduction intense enough to block any one of them may block a large proportion, if not all, at the same time; hence cardiac slowing is not likely to be gradual but abrupt, appearing first in the form of second stage heart block. Thus, rather than a fundamental difference in the nature of digitalis action on conduction, it is the characteristic irregularity of the intensity of the auricular impulses in auricular fibrillation which makes possible gradual ventricular slowing through a depressant action

of digitalis on atrioventricular conduction. This also makes it a very dependable clinical effect of digitalis.

Two elements enter into the complex mechanism of conduction depression by digitalis in auricular fibrillation just described; both require clarification. The first is the reflex slowing, confusingly named "vagal" slowing.[21, 26] This is really the same effect as that already described in the case of effective slowing of sinus tachycardia by heart failure; it is the consequence of the reduction of the accelerator stimulus following improvement in force of systolic contraction. As a result of this chain of events, the relatively normal high vagal tone of man tends to return. In auricular fibrillation, rather than on the sinoauricular node, the effective increase in vagal tone in this case is exerted on the atrioventricular conducting mechanism, thereby tending to depress conduction through the bundle of His. Thus, in auricular fibrillation, to the extent that the rapid ventricular rate is a response to a deficiency in myocardial contractile force and digitalis improves cardiac dynamics, it provides reflex slowing mediated through a vagal mechanism, *not through direct stimulation by digitalis of any part of the vagal apparatus,* but as a secondary consequence of its ionotropic action on the ventricle. Since this type of slowing involves vagal pathways, it can be abolished by the use of atropine as well as by exertion which brings accelerator mechanisms into play. As in the normal rhythm, this form of slowing does not develop at all if digitalis does not increase the force of contraction sufficiently to improve cardiac efficiency; it is always secondary to success in this respect and cannot occur without it. As already pointed out, in terms of mode of action, it is the same as the reflex slowing of the sinus tachycardia of heart failure. Neither does it occur if there is no heart failure with the auricular fibrillation, since there is no acceleration which is the result of a reflex that can be reversed by the ionotropic action of digitalis. It should therefore be called "reflex" slowing—occasionally it is. Unfortunately, it is more often called "vagal" slowing, a term which has implications of a particular site of digitalis action which do not really apply to this case.

The second component of slowing in auricular fibrillation is due to the direct action of digitalis on the conducting mechanism, most likely on the refractoriness of the atrioventricular node; this is often called "extravagal" slowing, a term used to contrast it with the "vagal" slowing and, consequently, equally unsatisfactory and really not as precise a term as "direct" slowing. This depressant action on conduction begins to develop at much the same time as reflex slowing but does not ordinarily become prominent until digitalization is well along; at about 80 per cent therapeutic digitalization and from then on, with continuing dosage, it becomes progressively dominant. In contrast to reflex slowing, which develops only when digitalis improves the efficiency of cardiodynamics, with appropriate dosage direct slowing can be induced in virtually all cases of auricular fibrillation. As might be suspected, direct slowing cannot be abolished with atropine or by other normal accelerator mechanisms, exertion for example.[40] It can be reversed only by digitalis elimination.

The importance of direct slowing in the treatment of heart failure in auricular fibrillation has been discussed at length in the literature. It is the stand of Gold and Cattell that direct slowing per se is of relatively less importance in the relief of heart failure and that the action of digitalis of greater moment is on the force of contraction of the ventricle. Not all are willing to agree, however, and there are some who contend that the slowing of the ventricular rate itself makes the greater contribution to heart failure by providing more time for myocardial recovery as well as for greater diastolic filling of the ventricle between cardiac contraction, hence, greater stroke output. The view of Gold and Cattell does not rule out assistance provided by slowing the heart but rather accepts it as a useful

concomitant of the ionotropic action of digitalis and the reflex slowing which ensues as a consequence. In support of their view is the clinical fact that the dosage of digitalis used in the treatment of heart failure with auricular fibrillation is rarely such that more than a small component of direct slowing is developed. In the very severely damaged heart, in which digitalis can induce little slowing through the reflex which ensues from its ionotropic action, direct slowing becomes a relatively more important matter. Since more digitalis is required for this action, it serves to explain the wider margin between therapeutic and toxic doses of mild than of severe heart disease.[8, 25] In most instances, however, whatever benefits accrue from the use of digitalis probably come only as the primary and secondary consequences of its ionotropic action, and, therefore, those who argue for the primary importance of cardiac slowing are calling for an action which, in most instances, is actually secondary to improvement in force of systolic contraction.

Slowing by digitalis in auricular fibrillation caused solely by the reflex action may be overcome by exertion, anxiety, or any other stimulant which normally tends to accelerate the heart or reduce vagal tone; direct slowing is not responsive to these stimuli. It is possible by adjustment of dose to obtain a suitable admixture of the two types of slowing to prevent the exaggerated acceleration characteristic of auricular fibrillation while preserving within acceptable limits an accelerator response to exertion. It is to be remembered, however, that slowing of the ventricular rate in auricular fibrillation by the direct action of digitalis alone may be suppressing an accelerator response to exertion which, in view of the failure of digitalis to improve cardiotonic action, should be retained so far as it may be useful.

The action of digitalis on conduction is a particularly sensitive as well as a reliable and useful one in the circumstance of auricular fibrillation, for the reasons given. The mechanisms involved make it possible to adjust dosage satisfactorily in most cases, although extended too far, excessive dosage may induce atrioventricular dissociation. The usual therapeutic doses depress conduction sufficiently to bring about clinically satisfactory ventricular rates with a normal sinus rhythm, however, conduction depression which slows or alters the heart rate constitutes a toxic effect of digitalis.

It is the rhythm which makes the difference.

### Cardiac output

There is no good experimental evidence in either the laboratory animal or normal man that digitalis exerts a specific effect on the myocardium to increase cardiac output or that it is capable of inducing supranormal output. Any influence digitalis may have on cardiac output in heart failure is secondary to actions already discussed—increased force of contraction and, in auricular fibrillation, through depression of conduction, slowing to permit greater cardiac filling.

### Venous pressure

In heart failure digitalis, when effectively used, lowers elevated venous pressure. There are experiments in the dog which indicate that digitalis has a direct action on the veins of the portal system to enhance cardiac filling. Unfortunately for man, however, he has little or none of the special sphincterlike musculature in his veins that enables digitalis to produce this effect in the dog, and this mechanism does not, therefore, apply to the effect of digitalis in man. The effect to lower the elevated venous pressure of heart failure is undoubtedly the consequence of increased force of systolic contractions and in man is certainly not due to a primary action of digitalis on the venous system. In the case of the dog with heart failure, it is conceivable that this action might play some part, but even in this species, the ionotropic action of digitalis is more likely to be the important one.

### Blood pressure

Digitalis has no significant effect on the arterial system in normal man or animals. Should its clinical use be followed by an elevation in blood pressure in man, it would undoubtedly be secondary to effects of digitalis on cardiodynamics already described. It is for this reason that, when its ionotropic action is needed, digitalis is equally safe and useful in both hypotension and hypertension.

### Coronary circulation

There is no substantial evidence of a specific effect of digitalis on coronary circulation; any effect is likely secondary to actions already described.

### Diuretic action

The sometimes dramatic diuretic action of digitalis in heart failure is usually correctly ascribed to increased renal blood flow and increased glomerular filtration following improvement in cardiodynamics; consequences of the actions on the heart discussed above. Because of the remarkable efficiency of tubular resorption of the glomerular filtrate, when the flow is decreased reabsorption may be virtually complete, and in this situation, even a very minute effect on cardiodynamics to increase glomerular filtration will markably augment the rate of urine flow. This effect of digitalis is, of course, not seen unless there is heart failure with edema.

There is also a direct renal action of digitalis which induces diuresis in the dog, and there is no evidence that it does not occur in man. This effect, which has been known for many years, is due to an action directly on the tubules to depress resorption of the glomerular filtrate. While it is of interest to students of the nature of diuresis, it is so feeble an effect that it could not possibly have practical importance in the actions of digitalis heart failure.

### Cardiac automaticity

In sufficiently large doses, digitalis reduces refractoriness of the myocardium and appears to stimulate cardiac irritability, to excite or promote the appearance of ectopic foci of rhythmicity in both man and animal.[12, 14, 15] These appear first as ventricular ectopic beats but, given enough digitalis, progress into ventricular tachycardia and fibrillation.[37] Heart block may develop somewhere along this course; its temporal relationship to the development of cardiac arrhythmias is not constant or predictable. Auricular fibrillation may also be induced with digitalis, but it is rarely identified. In these reactions to digitalis, there is no clinical or electrocardiographic difference between man and beast.

There is often some difficulty in determining in man whether premature beats are due to disease or digitalis. If the premature beat is identified when it first appears, hence just as the dosage reaches the excitant level, a day without digitalis should provide elimination sufficient to discover whether the ectopic foci are dependent on the presence of digitalis. The greater the overdosage, the longer the period of elimination necessary to judge the dependence of a disturbance in rhythm on digitalis. Although all rhythmic disorders are now considered toxic effects of digitalis, it should not be passed over that the induction of premature ventricular contractions was once used in the treatment of heart block to increase the otherwise very slow idioventricular rate, a practice which has long since given way to the use of the sympathomimetic amines. There is the interesting possibility in this connection that in heart block, since there is greater opportunity for re-entry, the ventricle is more responsive to the effect of digitalis on automaticity; thus the ventricle responds with premature contraction after doses of digitalis which are ordinarily considered to be nontoxic.

### Central nervous system

Digitalis exerts several effects on the central nervous system, emesis and visual disturbances, both toxic effects, being the most important clinically. Nausea and

vomiting occur in man and laboratory animals; it is not known whether visual disturbances occur in the latter.

The emetic action requires some clarification. Given orally, digitalis glycosides may cause nausea or vomiting through a local irritant action. This is considered a minor toxic effect. The incidence of this reaction appears to have decreased with the increased use of purified glycosides which, although also capable of a local emetic action, appear to be less irritating to the gastrointestinal tract than the unpurified Galenical compounds.

More ominous is vomiting due to central stimulation of the emetic trigger zone by digitalis.[5] This occurs as the consequence of absorbed digitalis; and insofar as it is usually the result of systemic poisoning and also of relatively large doses, it may presage other serious systemic effects.

Digitalis glycosides also exert convulsant or central excitant actions. Although rarely seen in other species, this is easily demonstrated in the rat and mouse. These animals convulse more readily after squills than after the other digitalis glycosides, but convulsions can be induced by the other glycosides as well.[28, 41] The apparent species specificity for a convulsant digitalis action may well be the consequence of the extreme (and species specific) cardiac tolerance of these rodents to digitalis glycosides which makes it possible for them to develop and demonstrate digitalis actions resulting from doses far exceeding those fatal for other species and to which they therefore cannot be exposed. Thus central excitement and convulsions are not ordinarily seen in other animals from glycosides. However, they have been observed in many species of animals after the aglycones (i.e., genins, the glycosides with the sugars removed) have been administered.

**Table I.** *Average doses of various digitalis preparations**

| | *Initial digitalization* | | *Daily maintenance* |
|---|---|---|---|
| *Preparation* | *Oral* | *Intravenous* | *Oral* |
| Digitalis | 1.2 Gm. | | 0.15 Gm. |
| Digitoxin | 1.2 mg. | 1.2 mg. | 0.15 mg. |
| Acetyldigitoxin | 2.0 mg. | 1.5 mg. | 0.3 mg. |
| Gitalin (amorphous) | 5.0 mg. | | 0.5 mg. |
| Digoxin | 3.0 mg. | 1.0 mg. | 0.5-0.75 mg. |
| Lanatoside C | 6.0 mg. | 1.6 mg. | 1.0 mg. |
| Ouabain | | 0.8 mg. | |
| Acetyl strophanthidin | | 0.6 mg. | |

*Adapted from Krantz, J. C., Jr.: Postgrad. Med. 24: 224, 1958.

### Parameters of digitalis action

It is usually assumed that for all practical therapeutic purposes, all the natural and semisynthetic digitalis materials, and there is a large number of them, have the same qualitative actions and that the important differences are quantitative ones which can be expressed in terms of pharmacologic parameters.[21, 32] The view that there are also qualitative differences and that some glycosides have cardiac actions not reproducible by proper adjustment of dose and mode of administration of any of the others is held only by a small minority of workers. As already mentioned, I hold the former view.

***Potency.*** Table I indicates the range of potency of several purified glycosidal materials. The Galenical compounds are considerably less potent, but, regardless of the weight of material necessary to induce a particular effect, since they are all qualitatively similar so far as cardiotonic effects are concerned, mere manipulation in dosage can biologically equate all differences in absolute potency.[27]

***Absorption.*** There are wide variations in absorption from the human gastrointestinal tract, as shown in Table II. Thus, while digitoxin is virtually completely absorbed, ouabain is absorbed not at all. The others lie somewhere between the two.[48]

***Development of action.*** Digitalis materials vary greatly in the rate of development of cardiac action.[13, 14] Thus, even after intravenous administration, digitoxin may take 6 to 10 hours for full development of effects, thevetin a few minutes, and ouabain about ½ hour. Fig. 2 indicates intermediate positions for some of the others. With other routes, the peak of the curve of development of action may be depressed by slow as well as incomplete absorption.

***Elimination.*** The mode of elimination of digitalis still is not yet known but differences in rate are well established for members of this series of drugs. At one extreme of the spectrum is digitoxin, which usually takes about 2 weeks for full elimination of a single digitalizing dose; at the other extreme is thevetin, which is completely eliminated in a matter of minutes. Fig. 2 indicates some of the intermediate members. It is of interest that although the biologic action may persist as long as 2 weeks, in the case of digitoxin the drug disappears chemically very promptly and its effects can be detected only by biologic means. This principle holds for other glycosides as well. So far as is known, digitalis not acting on the heart is not stored in tissues or body fluids but is completely eliminated in one way or another. There is, therefore, no basis for the once held view that in cases of marked diuresis there is danger from the liberation of digitalis from the edema fluid mobilized.

**Table II.** *Absorption of digitalis glycosides from the gastrointestinal tract*

| *Preparation* | *Approximate percentage* |
|---|---|
| Digitoxin | 100 |
| Digitalis leaf | 20 |
| Lanatoside C | 10 |
| Convallaria | 2 |
| Ouabain | Not absorbed |

***Curve of action.*** The continuous curves of development of action and elimination make up a time action curve which provides a thumbnail sketch of the development of drug effects (Fig. 2). This is the picture which will determine which drug is best suited to a particular situation, as well as the mode of administration and the dosage regimen for the maintenance of effective levels.[2, 6, 18, 24, 29, 30, 33, 42, 44] It is of special interest that, thus far, there seems to be no exception to a rule of symmetry:

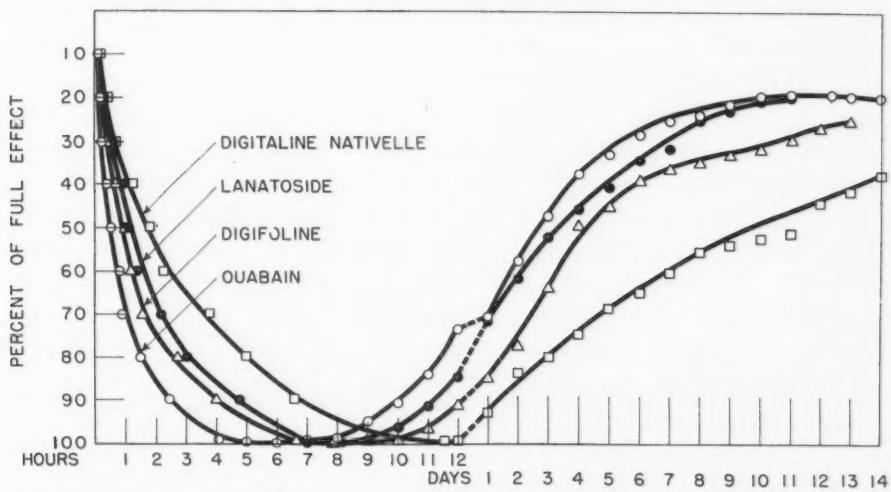



**Fig. 2.** The differences among digitalis glycosides in speed and duration of action after intravenous injection. Each curve represents the course of heart rate change after a single intravenous dose of 3 cat U. Each point on the curve represents the average of approximately 15 patients with auricular fibrillation. Note that ouabain produces full effects in about 2 hours and digitoxin (Digitaline Nativelle) in about 6 hours.

those which are slow to develop their effects are slowly eliminated, and those which induce effects quickly are also quickly eliminated.

***Dosage.*** From a practical point of view, this deals with the amount of glycoside necessary to produce a particular effect. Since there is no other way of estimating the results of a particular dose of digitalis, a biologic end point is essential in determining proper dosage with digitalis.[4, 23, 31]

The determination of the appropriate degree of digitalization requires careful consideration. The patient with auricular fibrillation has a built-in indicator of drug effect which, because of gradual slowing of the ventricular rate, continuously reflects intensity of digitalis action. Thus, merely by counting the ventricular rate, one can determine when a satisfactory degree of digitalization has developed in the patient with auricular fibrillation. All of Gold's statements on digitalis dosage are based on this handy and precise method of determining dosage effect.

Matters are not so simple when digitalis is used in patients with heart failure and a normal sinus mechanism. Here cardiac slowing does not necessarily parallel digitalis effect; consequently, the ventricular rate is also not dependably correlated with digitalis effect. Estimation of digitalis effect often has to be made on the basis of clinical improvement. This takes considerable clinical acumen and for purposes of any kind of clinical study is hard to collate quantitatively and in unbiased fashion. Since there is no reason why the action of digitalis on the myocardium is not precisely the same in all kinds of cardiac rhythms, Gold's observations on dosage of digitalis glycosides necessary to induce digitalization, determined in patients with auricular fibrillation, may be assumed to apply equally to patients with a normal sinus mechanism. This is consonant with the fact that in auricular fibrillation it is the ionotropic action of digitalis which is the major action in relieving heart failure and that in the normal rhythm therapeutic effects are completely dependent on the ionotropic action. These are, however, generalizations, and in each patient, whether or not there is a simple and convenient one like the response of the ventricular rate in auricular fibrillation to use as a guide, some clinical criterion of digitalis effect has to be used.

Two problems in dosage regimen are recognized: (1) initial digitalization and (2) maintenance.

*Initial digitalization.* This involves the establishment of the degree of digitalization needed therapeutically as rapidly as indicated by the circumstance and consistent with safety. Digitoxin will be used by way of example because of my own experiences with it.

A single dose approach obviously produces its full effects most rapidly. If it is an average dose which is used, i.e., a dose which will be effective in about half of the population (pharmacologically, an $ED_{50}$), by definition it will be an overdose or underdose for some of those who receive it. The number who receive considerably less and dangerously more will depend on the shape of the population distribution curve, that is to say, the range of deviation from the mean.

The initial digitalizing dose of digitoxin for the average patient has been stated by Gold to be 1.2 mg. This will satisfactorily initiate digitalization in about one-half of the population. It will cause vomiting only in about 3 per cent of those who take it at one time; slightly larger doses increase the incidence of emesis considerably. Some patients who receive a single dose of 1.2 mg. of digitoxin will not receive the full digitalizing dosage until they receive an additional 0.2 to 0.4 mg. of the drug on subsequent days; how much varies with the individual.

Any multiple dose method will obviously take somewhat more time to provide full digitalization but will also provide a means of trial in which to tailor the digitalizing dosage to fit the patient and the circumstance more precisely without the small

danger of overdosage present when using a single dose of 1.2 mg. routinely. The first dose in this regimen is usually the largest, generally 0.8 mg. of digitoxin. The incidence of vomiting is nil, which, for purposes of evaluating the method of digitalization, is to be compared with that of an initial dose of 1.2 mg. which induces vomiting in 3 per cent. Subsequent doses are usually 0.4 mg. increments given at 4 hour intervals until the desired effect is achieved. Eventually the same total dose as after the single dose of 1.2 mg. and the supplemental dose it may require will have to be used. Thus the essential differences between the multiple dose approach and the "single" dose method in which 1.2 mg. is given at one time are the 0.4 mg. amount of the first dose and the 4 hour delay in giving it. The 3 per cent chance of emesis is eliminated at the cost of 4 hours in achieving digitalization. Other multiple dose regimens will differ from the single dose regimen along the same lines. One has to make the choice on the basis of this difference. Since the emesis is never severe after 1.2 mg. of digitoxin, it is usually worth while to follow the single dose plan.

*Daily dose digitalization.* It is also feasible to digitalize by starting with the same dose, one equal to the maintenance dose, and continuing it on a daily basis.[25] In most instances, after about 3 weeks a dose of 0.15 to 0.2 mg. of digitoxin will induce full digitalization, after which effects will level off and not become more intense although the same dosage regimen is continued. This is so because digitalis is not eliminated by fixed daily amounts but by a fixed proportion of the total amount in the body. Therefore, no matter how small a daily dose is given, at first a proportion of it is left to accumulate. Eventually, when the dose taken daily equals in amount the proportion of the accumulated total excreted, digitalis no longer accumulates, the digitalis effects level off, and, although the same dosage is continued, no further accumulation can develop. Obviously, there will be individual differences in rate of elimination in each case which will determine the precise total dose and time of full digitalization. This method of digitalization had more merit years ago when, because of the irritant action of impurities, the incidence of emesis after use of the Galenical compounds was high. Today, with a low incidence of local emetic action because of greater use of purified glycosides; this method of digitalization is rarely indicated.

*Maintenance.* Maintenance dosage is merely a scheme to continue at an operative level a degree of digitalization achieved and considered to be desirable therapeutically. The amount of drug administered daily must be equivalent to the amount excreted or, depending on which is the larger, effects will either diminish or intensify. The principles on which this is based have been set forth above. The amount of digitalis eliminated daily relates to the amount present; it is not fixed. It must be replenished or effects will diminish or, if the daily dose is too large, will mount to toxic proportions. These are often insidious developments because they are gradual, and observations are not usually as close or as careful during maintenance as during initial digitalization. There seems to be far more concern with initial digitalization as a problem than with maintenance. As a consequence of this, perhaps, or because it is really a more difficult accomplishment which few view as such, serious intoxication is a more important complication of maintenance dosage than is generally recognized and, in my experience, a more frequent serious development than after initial digitalization. Failure of maintenance is even more common. It is well to remember that satisfactory, or level, digitalis maintenance must also take into account speed of excretion as well as amount. Since the digitalis glycosides differ greatly in this regard, the dosage regimen should vary with each glycoside. Thus, in the case of digitoxin, a single daily dose will provide satisfactory and even maintenance. This is so because roughly only 10 per cent of the dose in the body is ex-

creted daily. In the case of digoxin, however, of which as much as 33 to 50 per cent may be excreted daily, an adequate single daily replacement dose will produce overdigitalization at the beginning and underdigitalization at the end of the same day. Here, more satisfactory maintenance, that is, more level digitalization without toxic effects, may be obtained by dividing the daily replacement into three doses and spreading it over the day.[10]

*Toxicity and dosage.* Systemic cardiac toxicity is usually an extension of the therapeutic action of digitalis; one flows into the other as dosage is increased. They are not different actions. The ultimate which can be achieved therapeutically with digitalis is therefore a dose short of minor toxicity; the two need never coincide.[8] This standard was established by Withering in 1785 when he stated, ". . . let it be continued until it either acts in the kidneys, the stomach, the pulse, or the bowel; let it be stopped upon the first appearance of any one of these."[47] This is an operational procedure which calls for a dose so close to the toxic that were it to be applied as the basis for dosage for the average patient, it would most certainly be too large for some. It is, therefore, an inevitable consequence for some patients receiving a regimen which attempts to exploit the potential of digitalis to the fullest and is likely to occur in patients with a normal sinus rhythm, who do not have the sensitive indicator of digitalis possessed by those with auricular fibrillation. That Withering followed his own dictum was made clear to me one night during an insomniac period when instead of counting sheep I counted the number of minor toxic reactions in his original series. About 15 per cent of his patients had reacted with minor toxic symptoms. Surprisingly enough, this is the same incidence of toxicity in our experience with a large group of patients when 1.5 mg. digitoxin was given at one time. The incidence of gastrointestinal irritation and systemic intoxication can be reduced with modern preparations and multiple dosage regimens, but it is a virtual certainty that if the drug is adequately applied there will nevertheless be some reactions caused by overdosage. In clinics where there are none at all, the average dose is probably far too low. Serious reactions in the particular patient can always be avoided by careful dosage, but if every patient is to be given a chance to get as much benefit as he can from digitalis, since in severe heart disease the therapeutic range tends to narrow, some will certainly experience minor toxic symptoms.[8]

***Effects of calcium and potassium.*** It has been demonstrated in animals and proved in man as well that calcium and potassium affect the sensitivity of the myocardium to digitalis. Potassium deficiency and high blood calcium levels increase digitalis toxicity and, the reverse, elevated potassium and lowered blood calcium tend to decrease digitalis toxicity.[1, 3, 19, 20, 34-36, 43]

All devices and drugs which influence the level of these ions affect the response of the patient to digitalis. Potassium loss caused by chlorothiazide may intensify digitalis action; administering potassium may prevent or abolish digitalis arrhythmias.[16, 17] The administration of calcium intravenously may induce digitalis intoxication while the lowering of blood calcium by chelation with edathamil may reduce it.[11] The latter is the basis of a new digitalis tolerance test as well as a method of treatment of digitalis intoxication.

*Correlation and formulation.* It seems to me that it should be clear from the foregoing that there not only is a close correlation between the information on digitalis adduced through studies in the animal and the clinical observations of its effects in man, but there is also an explanation in the latter of how to use it well in the cat, the dog, and the horse with heart failure.

### *References*

1. Aikawa, J. K., and Rhoades, E. L.: Effects of digitoxin on exchangeable and tissue potassium contents, Proc. Soc. Exper. Biol. & Med. **90**: 332, 1955.

2. Aravanis, C., and Luisada, A. A.: Clinical comparison of six digitalis preparations by the parenteral route, Am. J. Cardiol. **1**:706, 1958.
3. Bellet, S.: Recent advances in the treatment of cardiac arrhythmias, Am. Heart J. **56**:479, 1958.
4. Bliss, C. I., Greiner, T., and Gold, H.: Estimating the dose of a cardiac glycoside for human subjects, J. Pharmacol. & Exper. Therap. **109**: 116, 1953.
5. Borison, H. L., and Wang, S. C.: Physiology and pharmacology of vomiting, Pharmacol. Rev. **5**:193, 1953.
6. Brill, I. C., Burgner, P. R., and David, N. A.: Acetyl-digitoxin (Acylanid): Rapid digitalization and maintenance by oral administration, Ann. Int. Med. **44**:707, 1956.
7. Cattell, M., and Gold, H.: Influence of digitalis glucosides on force of contraction of mammalian cardiac muscle, J. Pharmacol. & Exper. Therap. **62**:116, 1938.
8. Cattell, M., and Gold, H.: Studies on purified digitalis glucoside. III. The relationship between therapeutic and toxic potency, J. Pharmacol. & Exper. Therap. **71**:114, 1941.
9. Chen, K. K.: Pharmacology, Ann. Rev. Physiol. **7**:677, 1945.
10. Church, G., and Marriott, H. J. L.: Digoxin: Single versus divided daily maintenance dosage, Am. Heart J. **58**:171, 1959.
11. Cohen, B. D., Spritz, N., Lubash, G. D., and Rubin, A. L.: Use of a calcium chelating agent (NaEDTA) in cardiac arrhythmias, Circulation **19**:918, 1959.
12. Connolly, D. C.: Arrhythmias associated with digitalis therapy, Postgrad. Med. **25**:509, 1959.
13. Dayrit, C., Faraco, E., Kwit, N. T., Modell, W., and Gold, H.: Speed of action of thevetin after intravenous injection in man (Abst.), Fed. Proc. **7**, 1948.
14. Enselberg, C. D., Altchek, M. R., and Hellman, E.: The action of acetyl strophanthidin in rapid cardiac arrhythmias, Am. Heart J. **40**: 919, 1950.
15. Enselberg, C. D., Croce, J. P., Jr., and Lown, B.: Ventricular fibrillation due to digitalis preparations, Circulation **3**:647, 1951.
16. Fisch, C., Martz, B. L., Priebe, F. H., Steinmetz, E. F., and Chevalier, R. B.: Transient effect of potassium on A-V conduction and ventricular tachycardia produced by acetyl-strophanthidin: Demonstration of supranormal conduction, Am. Heart J. **60**:212, 1960.
17. Fisch, C., Martz, B. L., Priebe, F. H., Steinmetz, E. F., and Chevalier, R. B.: Transient effect of intravenous potassium on A-V conduction and ventricular ectopic beats, Am. Heart J. **60**:220, 1960.
18. Friedman, M., St. George, S., and Bine, R.: The behavior and fate of digitoxin in the experimental animal and man, Medicine **33**:15, 1954.
19. Garb, S., and Venturi, V.: The differential actions of potassium on the therapeutic and toxic effects of ouabain, J. Pharmacol. & Exper. Therap. **112**:94, 1954.
20. Gilbert, R., and Lyons, R. H.: Clinical evaluation of a digitalis tolerance test, Am. J. M. Sc. **232**:144, 1956.
21. Gold, H.: Pharmacologic basis of cardiac therapy, J.A.M.A. **132**:547, 1946.
22. Gold, H., and Cattell, M.: Mechanism of digitalis action in abolishing heart failure, Arch. Int. Med. **65**:263, 1940.
23. Gold, H., Cattell, M., Modell, W., Kwit, N. T., Kramer, M. L., and Zahm, W.: Clinical studies on digitoxin (Digitaline Nativelle): With further observations on its use in the single average full dose method of digitalization, J. Pharmacol. & Exper. Therap. **82**:187, 1944.
24. Gold, H., Cattell, M., Greiner, T., Hanlon, L. W., Kwit, N. T., Modell, W., Cotlove, E., Benton, J., and Otto, H. C.: Clinical pharmacology of digoxin, J. Pharmacol. & Exper. Therap. **109**:45, 1953.
25. Gold, H., and DeGraff, A. C.: Studies on digitalis in ambulatory cardiac patients. IV. Newer principles of digitalis dosage, J.A.M.A. **95**:1237, 1930.
26. Gold, H., Kwit, N. T., Otto, H., and Fox, T.: On the vagal and extravagal factors in cardiac slowing by digitalis in patients with auricular fibrillation, J. Clin. Invest. **18**:429, 1939.
27. Gold, H., Modell, W., and Otto, H.: Cumulative distribution curve of digitalis action in man by oral administration (Abst.), Fed. Proc. **4**, 1945.
28. Gold, H., Modell, W., Cattell, M., Benton, J. G., and Cotlove, E. W.: Action of digitalis glycosides on the central nervous system with special reference to the convulsant action of red squill, J. Pharmacol. & Exper. Therap. **91**: 15, 1947.
29. Gold, H., Modell, W., Kwit, N. T., Shane, S. J., Dayrit, C., Kramer, M. L., Zahm, W., and Otto, H. L.: Comparison of ouabain with strophanthidin-3 acetate by intravenous injection in man, J. Pharmacol. & Exper. Therap. **94**: 39, 1948.
30. Gold, H., Otto, H. L., Modell, W., and Halpern, S.: Behavior of synthetic esters of strophanthidin, the acetate, propionate, butyrate, and benzoate, in man, J. Pharmacol. & Exper. Therap. **86**:301, 1946.
31. Hauck, A. J., Ongley, P. A., and Nadas, A. S.: The use of digoxin in infants and children, Am. Heart J. **56**:443, 1958.
32. Krantz, J. C., Jr.: The cardiac glycosides in medical practice, Postgrad. Med. **24**:224, 1958.

33. Löffler, W., Essellier, A. F., and Forster, G.: Acetyl-digitoxin, Am. Heart J. **47**:898, 1954.
34. Lown, B., and Levine, S. A.: Current concepts in digitalis therapy, New England J. Med. **250**: 771, 1954.
35. Lown, B., Salzberg, H., Enselberg, C. D., and Weston, R. E.: Inter-relation between potassium metabolism and digitalis toxicity in heart failure, Proc. Soc. Exper. Biol. & Med. **76**: 797, 1951.
36. Lown, B., Wyatt, N. F., Crocker, A. T., Goodale, W. T., and Levine, S.: Inter-relationship of digitalis and potassium in auricular tachycardia with block, Am. Heart J. **45**:589, 1953.
37. McGee, R. R., and Tullis, I. F.: Ventricular tachycardia: With particular consideration of digitalis therapy, Am. J. Cardiol. **3**:300, 1959.
38. McLain, P. L., Kruse, T. K., and Redick, T. F.: The effect of atropine on digitoxin bradycardia in cats, J. Pharmacol. & Exper. Therap. **126**:76, 1959.
39. Modell, W.: The choice of drugs for diseases of the heart, *in* Drugs of choice 1960-1961, St. Louis, 1960, The C. V. Mosby Company, p. 392.
40. Modell, W., Gold, H., and Rothendler, H. H.: Use of digitalis to prevent exaggerated acceleration of heart during physical exercise in patients with auricular fibrillation, J.A.M.A. **116**:2241, 1941.
41. Modell, W., Kwit, N. T., Dayrit, C., Shane, S. J., and Gold, H.: Comparison of digitalis glycosides with their genins in man, Fed. Proc. **7**, 1948.
42. Modell, W., Kwit, N. T., Dayrit, C., Shane, S. J., Zahm, W., Kramer, M. L., and Gold, H.: Studies on purified digitalis glycosides, J. Pharmacol. & Exper. Therap. **94**:44, 1948.
43. Regan, T. J., Christensen, R. C., Wada, T., Talmers, F. N., and Hellems, H. K.: Myocardial response to acetyl strophanthidin in congestive heart failure: A study of electrolytes and carbohydrate substrates, J. Clin. Invest. **38**:306, 1959.
44. Soloff, L. A., and Zatuchni, J.: Clinical experiences with digoxin, Am. Heart. J. **57**:674, 1959.
45. Stoll, A.: The cardiac glycosides, London, 1937, The Pharmaceutical Press.
46. Turner, R. B.: Structure and synthesis of cardiac genins, Chem. Rev. **43**:1, 1948.
47. Withering, W.: An account of the foxglove and some of its medical uses; with practical remarks on dropsy and other diseases, Birmingham and London, 1785; reprinted *in* Medical classics, vol. 2, Baltimore, 1937, Williams & Wilkins Company.
48. Wright, S. E.: The metabolism of cardiac glycosides, Springfield, Ill., 1960, Charles C Thomas, Publisher.

# Clinical pharmacology of antithyroid compounds

*Successful treatment of hyperthyroidism is predicated upon an understanding of the pharmacology of antithyroid drugs as well as upon a knowledge of their effects on pathways of thyroidal iodide metabolism. Research into the relationship between chemical structure and biologic action of numerous antithyroid compounds has served as a basis for their classification and for selection of those of greatest potency for clinical trial. Biochemical determination of the site and mode of action of compounds affecting thyroid hormonogenesis, although incomplete, allows a tentative working hypothesis which must be periodically revised as further evidence is accumulated. During the 17 years since the introduction of thiouracil, clinical pharmacologic investigations have established optimal dosage schedules, the incidence of toxic reactions, and the indications for the effective use of these drugs. Such studies have further served to develop programs for the management of patients with hyperthyroidism with special emphasis on the use of antithyroid compounds in long-term therapy, preoperative preparation, and use during childhood or pregnancy. Although refinements in surgical approach and the general availability of radioactive iodine have necessitated modifications in the indications for their use, antithyroid drugs still maintain a secure position as therapeutic agents in the treatment of hyperthyroidism.*

**Herbert A. Selenkow, M.D., and Fernando Magalhães Collaço, M.D.**
*Boston, Mass.*
*Department of Medicine, Harvard Medical School, and the Peter Bent Brigham Hospital*

The clinical introduction of thiouracil by Astwood in 1943 was followed by extensive investigation of numerous chemical compounds for their antithyroid properties. Because of either limitations in effectiveness or toxicity, only a few have achieved therapeutic importance and are currently employed in the treatment of hyperthyroidism. The remainder have ably served to characterize the relationship between chemical configuration and antithyroid activity and to assist in the elucidation of various biochemical and physiologic processes in thyroid hormonogenesis.

The term "antithyroid compound," in its generic sense, denotes all chemical substances which inhibit the synthesis or action of thyroid hormones. This may be accomplished by (1) interruption of pathways in the biosynthesis or the release of thyroid hormones, either directly or indirectly, (2) interference with the plasma or membrane transport of thyroid hormones, or (3) inhibition of the peripheral action of thyroid hormones at a cellular or molecular level. Common usage, however,

Received for publication Dec. 1, 1960.

limits the term "antithyroid compounds" to substances which act in the thyroid gland to interfere with the production or secretion of thyroid hormones. This latter definition includes the clinically useful compounds currently employed in the treatment of hyperthyroidism.

Antithyroid compounds designated as "goitrogens" derive this descriptive name from the fact that when administered in appropriate doses to intact animals or man, they produce enlargement of the thyroid gland. The mechanism of this enlargement is particularly pertinent since it has been determined that, at least for the most part, goitrogenesis results from pituitary thyrotropin stimulation. In the postulated sequence of events, goitrogenic compounds interfere with the synthesis or release of thyroid hormones, causing a diminution of the level of these hormones in blood and tissues. Responding to this deprivation, the pituitary gland secretes thyrotropin to stimulate the thyroid in an effort to increase hormone production and to restore homeostasis. However, if the chemical block remains, the result of persistent thyrotropin secretion on the thyroid gland is goiter formation without hormonal synthesis. Goitrogenesis can be prevented or reversed by hypophysectomy or by the administration of thyroid hormones or thyromimetic compounds which inhibit thyrotropin secretion.

It is rather difficult, in retrospect, to appreciate fully the impact which the introduction of antithyroid compounds had upon the treatment of hyperthyroidism. Prior to 1923, surgical mortality in patients with hyperthyroidism was prohibitive. The introduction by Plummer[131] of the preoperative use of iodides reduced this mortality significantly, but the levels were nevertheless still far from permissible, by current medical standards. The development of the drug therapy of hyperthyroidism and the striking improvements in surgical mortality and morbidity rates consequent to the clinical introduction of thiouracil are now legend. For many, surgical operation was no longer mandatory. For those in whom it was indicated, antithyroid compounds permitted refinements in technique which diminished surgical hazards to an almost irreducible minimum. The medical therapy of hyperthyroidism has indeed been one of the milestones in the progress of medicine.

During the past 17 years, antithyroid drugs have achieved an established place in clinical therapeutics. Their usefulness and applicability, however, have undergone modifications as a result of great experience with their use, introduction of radioactive iodine, and refinements in surgical concepts and techniques. The purpose of this review is to summarize the current status of the clinically important antithyroid compounds in order to place them in proper perspective with other available therapeutic measures and to emphasize certain biochemical, pharmacologic, and therapeutic considerations which are particularly germane to their effective utilization.

### Historical background

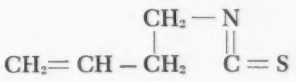
Recognition of the pathologic significance of thyroidal enlargement dates back to early historic times. However, discovery of the goitrogenic properties of diets other than those deficient in iodide must be credited to Chesney, Clawson, and Webster,[38] who observed that rabbits fed cabbage developed hyperplastic goiters. Although this was not due to a deficiency of iodide, goiter could be prevented by its administration. It was presumed that this diet contained a goitrogenic compound. Daily administration of large quantities of iodide to animals rendered goitrous by diets containing antithyroid substances produced marked weight loss and death, suggestive of "Jod Basedow."[116, 182] Marine and colleagues[117] investigated the thyroidal effects of isothiocyanates and demonstrated that acetonitrile and related compounds were goitrogenic and that their effects on the thyroid could be blocked by iodide administration. Hercus and Purves[87] discovered that rape

and cabbage seeds were goitrogenic agents in rats.

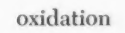
Experimental recognition of the goitrogenic properties of specific chemical substances was made fortuitously by MacKenzie, MacKenzie, and McCollum[115] while they were investigating synthetic factors in bacterial growth. They observed that sulfaguanidine produced goiters in rats fed a highly purified diet. Further investigations into the chemical configuration responsible for this effect revealed that both sulfanilamide and thiourea were potent goitrogens in rats, mice, and dogs.[114] That same year, Richter and Clisby[142] studied the toxicity of bitter-tasting phenylthiocarbamide (phenylthiourea) and noted that this substance produced hyperplastic goiters. MacKenzie and MacKenzie[113] made the important observation that unlike all previous experimental goiters, those produced by the sulfonamides and thiourea derivatives were associated with hypothyroidism and were not prevented by even massive doses of iodide. They concluded that these chemical substances produced goiter and hypothyroidism by suppression of thyroid hormone synthesis. That the pituitary was involved in the production of thyroid hyperplasia was shown by Kennedy and Purves,[82, 94] who demonstrated that the goitrogenic effects of rape seed could be abolished by hypophysectomy but not by iodine administration. This was later confirmed by the work of MacKenzie and MacKenzie[113] and of Astwood.[9] It was also demonstrated that administration of thyroxine to intact, drug-treated animals could suppress goiter formation completely.[113]

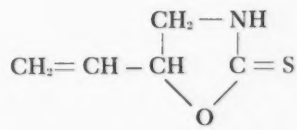
Clinical application of these experimental observations was initiated by Astwood,[10, 11] who showed that thiourea and thiouracil were effective in the therapy of hyperthyroidism. Numerous compounds of diverse chemical structure have been investigated over the subsequent years, and their antithyroid properties have been determined.[3, 13, 28, 104, 111, 137, 143, 167, 173, 176]

The goitrogenic substances contained in the naturally occurring members of the Brassica family have continued to interest investigators. In 1949, an active substance was isolated from rape seed by Astwood, Greer, and Ettlinger[19] which was identified as L-5-vinyl-2-thio-oxazolidone. This compound was also found in cabbage, kale, and yellow turnips. Greer[80] demonstrated that this compound was present in seeds and roots in an inactive form (progoitrin) which required enzymatic action for conversion to its active form (goitrin). Libermann[101] suggested that the inactive precursor was Δ-3-butenyl isothiocyanate, which is known to be present in many cruciferae, especially rape seed. The oxidative conversion may be represented as follows:



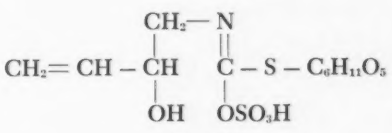
Δ-3-Butenyl isothiocyanate



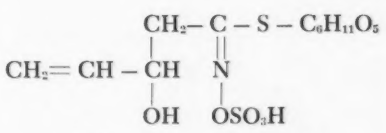


L-5-Vinyl-2-thio-oxazolidone

Greer[80] suggested a formula for progoitrin; however, subsequent investigations by Ettlinger and Lundeen[61] have shown the structure of progoitrin to be slightly different from that submitted by Greer:



*Greer*



*Ettlinger and Lundeen*

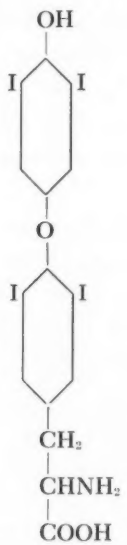
Progoitrin

An excellent review by Clements of the naturally occurring goitrogens has recently appeared.[42] A firsthand summation of the historical background of both natural and synthetic antithyroid compounds has been written by MacKenzie.[112]

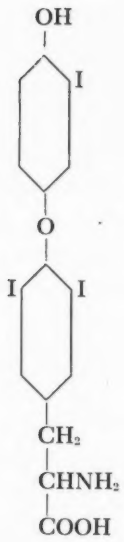
### Physiologic and biochemical actions of antithyroid drugs

An understanding of the action of chemical compounds on thyroid hormonogenesis must be predicated upon a basic knowledge of the physiologic pathways of iodide metabolism. It is pertinent to review briefly the fundamental steps in the biosynthesis of thyroid hormones in order to indicate the sites and modes of action of antithyroid drugs.

***Intrathyroidal pathways of iodide metabolism.*** Inorganic iodine entering the circulation from the gastrointestinal tract or from other sources exists in the reduced ionic form as $I^-$. This iodide is distributed throughout the extracellular fluid compartment in a manner similar to chloride and is ordinarily present in plasma of normal subjects in amounts of the magnitude of 1 $\mu$g per 100 ml. Iodide is selectively removed from plasma by the thyroid gland for synthesis of the thyroid hormones L-thyroxine and 3,5,3′-L-triiodothyronine:




3,5,3′,5′-L-Tetraiodothyronine
(thyroxine)




3,5,3′-L-Triiodothyronine
(liothyronine)

The application of tracer studies using radioactive iodide, of newer techniques of chromatography and radioautography, and of the chemical analysis of iodide have permitted a systematic characterization of thyroidal iodide metabolism. This subject has been well reviewed in detail in several recent articles.[85, 119, 129, 130] The following is a brief, simplified presentation of some of the basic aspects of thyroid hormone synthesis to assist in an understanding of the action of antithyroid compounds.

The thyroidal synthesis of thyroxine and triiodothyronine, the metabolically important thyroid hormones, may be divided into four fundamental reactions:

I. Trapping:

$$I^- \text{ (plasma)} \underset{\text{gradient}}{\overset{\text{energy}}{\rightleftarrows}} I^- \text{ (thyroid cells)}$$

II. Binding:

$$I^- \rightarrow (I^\circ) \rightarrow \text{monoiodotyrosine (MIT) and diiodotyrosine (DIT)}$$

III. Coupling:

$$2 \text{ DIT} \longrightarrow \text{thyroxine } (T_4)$$
$$\text{MIT} + \text{DIT} \rightarrow \text{triiodothyronine } (T_3)$$

IV. Releasing:

$$T_4 \text{ and } T_3 \text{ (thyroglobulin)} \xrightarrow[\text{hydrolysis}]{\text{enzymatic}} T_4 \text{ and } T_3 \text{ (plasma)}$$

*Trapping: Selective concentration of iodide above serum levels.* The unique ability of the thyroid gland to collect and concentrate iodide above plasma levels has been termed "trapping." There is thus created a "gradient" between plasma and thyroidal iodide, the magnitude of which varies with the avidity of the thyroid gland for iodide. It is enhanced by pituitary thyrotropin stimulation and perhaps by the level of thyroidal iodide stores. Trapping of iodide is related to, but independent of, the organically bound iodide in the thyroid; it appears to be enzymatically mediated and oxygen dependent.[154] The factors influencing iodide trapping and clearance from plasma have been reviewed by Berson.[25, 193]

The simplest way to isolate and study the trapping mechanism is in hyperthyroid subjects in whom organic binding of iodide is blocked by administration of organic antithyroid compounds. This affords an opportunity to demonstrate the site and mode of action of the inorganic goitrogens as exemplified in Figs. 1 and 2. Both potassium perchlorate ($KClO_4$) and potassium thiocyanate (KSCN) inhibit the accumulation of $I^-$ above the plasma level by the thyroid gland. They accomplish this by "paralyzing" the mechanism responsible for maintenance of the increased thyroid: plasma gradient, thus preventing concentration of iodide by the thyroid gland beyond those levels present in plasma. Moreover, inhibition of the mechanism necessary for maintenance of the gradient will then permit discharge of any iodide trapped by the thyroid but not yet organically bound. Iodide will thus be returned to the plasma until the concentration in plasma and thyroid is essentially equal (Fig. 2). Although both perchlorate and thiocyanate ions can accomplish inhibition of the thyroid:plasma gradient, perchlorate is by far the more potent compound.[196] Also, these anions appear to accomplish their effects via different mechanisms, since thiocyanate is metabolized by thyroid gland slices[194, 195] whereas perchlorate remains unaffected chemically.[2] Thyrotropin has the opposite effect of thiocyanate or perchlorate, since it exerts a marked effect on enhancing the trapping mechanism; contrariwise, hypophysectomy markedly reduces but does not eradicate trapping.

The direct action of iodide on the inhibition of trapping is a complex problem which has not yet been clearly delineated. Iodide certainly has a pronounced effect on the uptake of $I^{131}$ by the thyroid gland. This phenomenon is commonly seen in patients or animals receiving amounts of iodide which increase the total body pool. However, a diminished radioactive iodine uptake does not necessarily indicate inhibition of the trapping mechanism. Radioactive iodine uptake is not a measure of the total iodide trapped but simply represents the *percentage* of the total iodide pool accumulated in a given period of time.[4] There is, however, evidence that iodide may inhibit trapping, at least indirectly, via two mechanisms: (1) by inactivation or inhibition of thyrotropin[1] and (2) by the effects of iodide on blocking organic binding or releasing.[36, 81] The organic goitrogens, such as methimazole, do not appear to interfere directly with trapping but instead diminish the total uptake of radioactive iodine by the thyroid gland by interfering with the subsequent steps of synthesis.

Iodide is competitive with thiocyanate and perchlorate. Administration of iodide can reverse the block created by these ions and permit organic binding. This is clinically demonstrated by reversal of control of thyrotoxicosis in patients receiving perchlorate who are given iodide in preparation for subtotal thyroidectomy.[74] It further explains why the dose of potassium perchlorate necessary to control thyrotoxicosis is dependent upon the level of dietary iodide and why optimal antithyroid effect is accomplished by either low iodide intakes or administration of large doses of perchlorate.[46]

The first observation of the antithyroid properties of potassium thiocyanate was

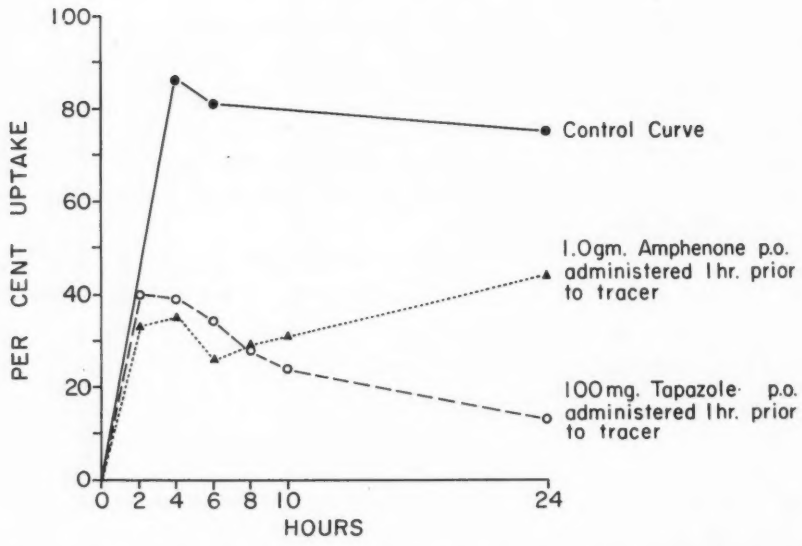



**Fig. 1.** The effect of a single oral dose of 100 mg. mercaptoimidazole (Tapazole), represented by the curve of open circles, or 1 Gm. amphenone (3,3′-bis[*p*-aminophenyl]-2-butanone), represented by the curve of solid triangles, on the radioactive iodine uptake of a hyperthyroid subject. The curve of solid dots is the control uptake prior to administration of either compound. Amphenone[151] is an organic goitrogen containing two aniline groups. Note that at 2 to 4 hours after mercaptoimidazole or amphenone, the thyroidal uptake of radioactive iodine is markedly reduced. All the accumulated $I^{131}$ at that time is trapped and is readily dischargeable (Fig. 2). At 24 hours, the blocking effects of amphenone have subsided, permitting reaccumulation of radioactive iodine, whereas the blocking effects of mercaptoimidazole on thyroid uptake are still quite apparent.

made in 1936 by Barker,[21] who found that several patients being treated with thiocyanate for hypertension developed signs of myxedema associated with enlargement of the thyroid gland. This effect could be rapidly reversed by the administration of small doses of thyroid or iodide.

It was shown in 1946 that thiocyanate markedly inhibits the accumulation of iodide by the thyroid gland.[174, 192] Vanderlaan and Vanderlaan[177] further demonstrated that as little as 1 mg. of potassium thiocyanate causes immediate discharge of inorganic trapped iodide, a phenomenon which can also be demonstrated in man through the utilization of radioactive iodine.

There are recent reports of goitrous children with hypothyroidism who, because unable to trap radioactive iodine, are unable to produce thyroid hormones.[29, 62] They may exemplify the clinical counterpart of the absence of a mechanism requisite for concentrating iodide, a congenital defect analogous to the inhibition of trapping in normal subjects by thiocyanate or perchlorate.

*Binding: Conversion of iodide to iodotyrosine compounds.* In normal thyroid glands, iodide in the thyroid trap is almost instantaneously converted into organic form (bound). Only a small amount appears to be present as iodide in the gland at any time. The process of binding involves oxidation of iodide to an intermediate state which has the oxidative potential essential for the iodination of tyrosine. This step seems to be accomplished by oxidative enzymes, perhaps peroxidases. However, the particular chemical structure of the iodine intermediate is unknown. It may be either free iodine ($I^{\circ}$) or hypoiodite, ($IO^{-}$) which, in the presence of tyrosine contained in thyroidal proteins, immediately forms monoiodotyrosine. Evidence favors the theory that this occurs in the

thyroidal proteins or peptides and not with free tyrosine.[170, 171] It has been clearly shown that most proteins containing tyrosine are readily iodinated in vitro. Once the iodide is fixed in the form of monoiodotyrosine, it can no longer be discharged from the thyroid by thiocyanate or perchlorate. This fixation process is a convenient way for the thyroid gland to store iodide, since approximately 50 per cent of the thyroidal iodide is present as iodotyrosines. Further iodination of monoiodotyrosine rapidly follows to form diiodotyrosine.[130]

The action of antithyroid compounds on this phase of iodide metabolism is particularly interesting. A great body of evidence indicates that organic goitrogens, such as thiouracil derivatives, prevent hormonal synthesis by interference with some phase of binding. However, it is still not clear whether they affect only oxidation of iodide or all steps through or even beyond the formation of iodotyrosines.[140, 155] It was previously observed that when binding of iodide is blocked by propylthiouracil, the thyroid:serum iodide ratio steadily increases (because of thyrotropin stimulation) and that all the thyroidal iodine is present as iodide.[21] More recently, however, some monoiodotyrosine and a smaller amount of diiodotyrosine have been detected in thyroid glands presumably completely blocked. Definitive resolution of which stage or stages in this binding phase are blocked by the organic goitrogens must await further investigations.

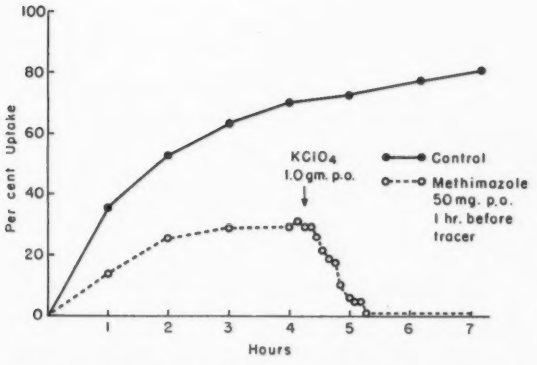



**Fig. 2.** The effect of potassium perchlorate on the trapped thyroidal radioactive iodide in a patient with hyperthyroidism. The curve of solid dots represents the $I^{131}$ uptake prior to any medication. Methimazole 50 mg. by mouth was given 1 hour prior to determination of the curve represented by open circles. At 4 hours, a single oral dose of 1 Gm. potassium perchlorate was given. Almost immediately, the radioactive iodide was rapidly discharged from the gland, indicating that it was present as trapped iodide and was not organically bound.

The mechanism by which the organic goitrogenic compounds accomplish inhibition of iodide binding has been extensively studied.[14, 16, 128] It was originally postulated that the sulfhydryl group, which is strongly reducing, inhibits oxidation of iodide and thus blocks iodination of tyrosine. Were this so, their action should be overcome by excessive iodide administration. This concept also fails to account for the goitrogenic effects of resorcinol or the aminocyclic compounds. Evidence reviewed by Astwood[16] supports the view that these compounds act by inhibition of the oxidizing enzymes involved in organic binding. The difference between these two concepts is not great, because both support failure of formation of the iodotyrosines from iodide.

This phase of thyroid hormonogenesis is defective in situations other than administration of goitrogenic substances. There is a group of goitrous cretins in whom a developmental defect in the iodination of tyrosine has been demonstrated, presumably on the basis of congenital absence of the appropriate oxidative enzyme systems.[41, 71, 161, 162] The thyroids of these children are capable of trapping large quantities of iodide but are unable to bind it by synthesizing iodotyrosines. This results in goiter and cretinism caused by inability to complete the synthesis of thyroid hormones. The low level of thyroid hormones in the blood stimulates secretion of thyrotropin in an effort to increase iodotyrosine synthesis but, in view of the inherent defect, results only in goiter formation and a consequent high avidity for trapping iodide.

The increased trapping of iodide in these children is comparable to that of thyroid glands of hyperthyroid subjects under block by organic goitrogens such as propylthiouracil or methimazole. This iodide is readily dischargeable by thiocyanate or perchlorate ions, a phenomenon which is of assistance in validating the site of the inherited defect.

*Coupling: Condensation of iodotyrosines to form iodothyronines.* The monoiodotyrosine and diiodotyrosine stored in thyroid colloid serve as a ready reserve for the basic constituents requisite for synthesis of thyroid hormones. By chemical condensation (coupling) of iodotyrosines in protein linkage, the diphenyl ethers, thyroxine and 3,5,3′-triiodothyronine, are formed. In addition, small quantities of other iodothyronines may accompany this reaction.[145] That this coupling mechanism is the route by which these hormones are synthesized was clearly shown in 1939 by von Mutzenbecher,[180] who demonstrated the formation of thyroxine from iodinated casein in vitro. Pitt-Rivers[127] pointed out that certain N-substituted derivatives of diiodotyrosine yielded significant amounts of the corresponding thyroxine derivatives during simple aerobic incubation at biologic pH. It may be unnecessary to assume an enzymatic mechanism for a reaction which occurs so easily in vitro.[128] Morton and associates[122] demonstrated that in hypophysectomized animals the conversion of iodide to diiodotyrosine is not greatly inhibited, but the conversion of diiodotyrosine to thyroxine is depressed to a considerable extent, thus hinting at the possible existence of another enzymatic system unable to function in the absence of thyrotropin. Pitt-Rivers,[128] however, has suggested two other mechanisms to explain these findings: (1) since the amount of iodide concentrated by the thyroid was greatly diminished by hypophysectomy, perhaps there was not enough free iodide (or hypoiodite) left for the coupling of diiodotyrosine, or (2) the diiodotyrosine molecules themselves might be too widely scattered throughout thyroglobulin to permit their access to each other for coupling.

Richards and Ingbar[140] reported that administration of increasing doses of propylthiouracil leads to a progressive reduction in the total $I^{131}$ uptake and organic binding. Furthermore, the MIT:DIT ratio increases and the amount of labeled thyronines becomes undetectable. No decrease in the ratio T-4:T-3 was noted. They state that propylthiouracil exerts an inhibitory effect upon the deiodination of MIT and perhaps more specifically upon the coupling of MIT or DIT to form iodothyronines. These effects would arise from the action of propylthiouracil upon the synthesis of thyroid proteins or upon the molecular rearrangements incident to the coupling process. This is a possible explanation for the restoration of a eumetabolic state in hyperthyroid patients receiving propylthiouracil whose uptakes of $I^{131}$ remain abnormally high. The antithyroid drugs probably not only diminish the total quantity but also alter the chemical nature of the organically bound iodine within the gland favoring the synthesis of hormonally inactive products.

A congenital defect in the ability of the thyroid to couple iodotyrosine radicals was first reported by Stanbury, Ohela, and Pitt-Rivers[164] and later by others.[100, 123, 184] It is not possible now to substantiate completely whether this inborn error of thyroid hormone synthesis is caused by absence of a "coupling enzyme" or by an abnormal steric configuration of thyroglobulin.[155]

*Releasing: Secretion of thyroid hormones into the circulation.* Thyroidal colloid (thyroglobulin) serves as an available reservoir for mono- and diiodotyrosine, thyroxine, and triiodothyronine bound in peptide linkage, in addition to small amounts of iodide and iodohistidine. To secrete only thyroxine and triiodothyronine into the circulation under exquisitely regulated conditions while at the same time retaining mono- and diiodotyrosine along with iodide and iodohistidine requires a precise and integrated sys-

tem. Such is the releasing mechanism of the thyroid gland. Proteolytic enzymes present in the thyroid gland hydrolyze thyroglobulin and thus liberate all iodinated amino acids. These enzymes are under the control of thyrotropin, which increases their activity as required to maintain a fixed blood or tissue level of thyroid hormones. In opposition to this, large quantities of iodide inhibit the release of thyroid hormones and favor the accumulation of colloid.

The thyroid secretion of mono- and diiodotyrosine, neither of which is calorigenically active, is prevented by an enzymatic mechanism designated as "tyrosine desiodinase." This enzyme is capable of preferentially deiodinating mono- and diiodotyrosine but not thyroxine or triiodothyronine. The liberated iodide can be recirculated via the intrathyroidal cycle to be stored for further organic binding. Valuable reservoirs of iodide are thus conserved. The thyroid hormones have been clearly shown to circulate as free amino acids which associate with the plasma proteins.[96] Under physiologic conditions, neither thyroglobulin nor iodinated peptides enter the circulation.

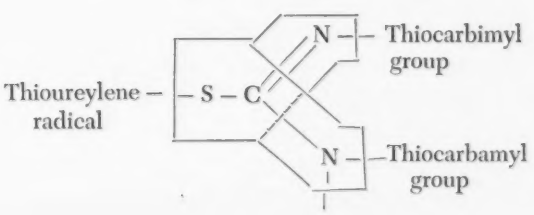
There is no evidence that any antithyroid compounds act directly upon the releasing mechanism. They may affect it indirectly by inducing increased thyrotropin secretion and subsequent goitrogenesis. The administration of both an organic goitrogen such as thiouracil plus iodide will permit the accumulation of colloid in the thyroid gland, but this colloid is distinctly deficient in organic iodinated amino acids.[138]

Iodide appears to have a specific effect upon the release of thyroid hormones. This phenomenon has been investigated by several groups.[75, 81, 156] In euthyroid subjects and in the absence of exogenous thyrotropin, iodide alone has no demonstrable effect on the release of thyroid hormones as measured by radioiodide. However, stimulation of the gland by thyrotropin permits demonstration of an effect of iodide on diminishing discharge of radioiodide.[55] In hyperthyroid subjects, the exhibition of as little as 1 mg. of iodide daily alters the secretion rate. The induction of myxedema in occasional euthyroid patients receiving large quantities of iodide[125] and the beneficial therapeutic effects of iodide upon hyperthyroid subjects may be dependent upon iodide inhibition of hormonal release.

Inborn defects in deshalogenation have been described in goitrous cretins.[166] The thyroid glands are unable to deiodinate mono- and diiodotyrosine, and consequently iodotyrosines are secreted into the circulation along with thyroxine and triiodothyronine. Large quantities of iodotyrosines are found in the urine of these children and may contribute to a persistent loss of iodide from the body.[109, 163] Tissue obtained by biopsy of the thyroid glands[136] as well as from other organs[39] fails to deiodinate mono- and diiodotyrosine in vitro. The entire subject of inborn errors of thyroid hormonogenesis has recently been reviewed.[108, 165]

### Pharmacology of antithyroid drugs

***Classification and chemistry.*** Numerous structurally related and unrelated chemical compounds have been investigated for their antithyroid properties. Those which are effective include simple inorganic ions as well as complex straight chain and cyclic organic moieties. It has been difficult to derive a clear relationship between chemical structure and biologic activity. Astwood[9] has studied a large number of goitrogenic substances and classified those of organic structure into two major categories: (1) chemical compounds containing an amino-substituted aromatic nucleus such as paraminobenzoic acid or the sulfonamides and (2) a larger group containing the thioureylene radical.



This latter group includes most of the potent antithyroid compounds and those of major therapeutic importance. It has since become necessary to enlarge the first category to include isocyclic and heterocyclic amino compounds and to modify the second category to include thiocarbamyl and thiocarbimyl groups, which together constitute the thioureylene radical. A third group of miscellaneous compounds containing neither nitrogen nor sulfur must now also be included. Thus, a revised chemical classification is as follows:

I. Inorganic antithyroid compounds
II. Organic antithyroid compounds
   a. Aminobenzene and aminoheterocyclic derivatives
   b. Thiocarbimyl and thiocarbamyl derivatives
   c. Miscellaneous compounds

*Inorganic antithyroid compounds.* Thiocyanate compounds are known to be goitrogenic in man and in animals. Of greater importance than inorganic thiocyanates are the organically bound isothiocyanates of mustard seed oils, the organic nitriles, and the cyanogenic glucosides which are converted into active goitrogens in mammals.[14] The isothiocyanate compounds are found in abundance in nature among plants of the Brassica and Umbelliferae families. The exact role of these substances in the etiology of endemic goiter is not clear, but they probably do not have a major influence except in areas with reduced dietary iodine.

Those naturally occurring substances which contain thiocyanate or are converted to it belong to the inorganic group of goitrogens and do not cause goiter when there is an abundance of iodide in the diet. However, goiters induced by the group of naturally occurring substances which are oxidized to organic heterocyclic antithyroid compounds (such as those described by Astwood[19]) are not influenced by iodide since their actions are comparable to the organic group of antithyroid substances.

More recently, other inorganic anions have been studied for their goiter-producing properties. Wyngaarden[196] found that perchlorate caused effects similar to thiocyanate, but that it was a much more potent goitrogen. Because of its potency, it has become an effective antithyroid compound in the treatment of hyperthyroidism. Other inorganic anions found to be weakly active were chlorate, iodate, biiodate, hypochlorite, periodate, and nitrate.[197]

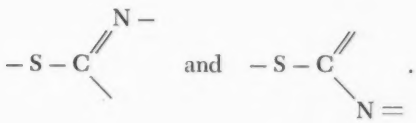
Cations such as cobalt have been reported to cause goiter, especially in children, but the exact mechanisms for this are not clear.[92, 124, 144] A discussion of the goitrogenic properties of metallic ions will not be included here. Similarly, discussion of the antithyroid properties of iodide and the relationship of iodide deficiency to goiter production are beyond the desired scope of this review. The interested reader is referred to several reviews on iodide and its relation to goitrogenesis.[58, 59, 135, 146] The influence of iodide on the physiologic status of the thyroid and on other goitrogens will be discussed under the appropriate sections.

*Organic antithyroid compounds.*

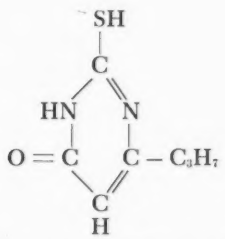
AMINOBENZENE AND AMINOHETEROCYCLIC COMPOUNDS. This interesting group of weak but clinically important antithyroid compounds includes numerous drugs used therapeutically for nonthyroidal disorders. These medications share the amino substituted aromatic or cyclic nucleus. Of particular importance are paraminobenzoic and paraminosalicylic acids, which have appreciable antithyroid activity. Indeed, goiters and hypothyroidism are not uncommon in tubercular patients who receive large amounts of paraminosalicylic acid.[20] Moreover, paraminobenzoic acid has been used clinically in the treatment of thyrotoxicosis.[80] Although these compounds are not sufficiently effective to be useful in current therapy, the goitrogenic side effects should be carefully observed. The sulfonamide drugs which are mildly goitrogenic in animals have not to date exhibited significant effects in man. Some members of

the related group of hypoglycemic sulfonylurea compounds, especially carbutamide, interfere with the thyroidal uptake of radioactive iodine.[139] The major significance of the thyroidal effects of these compounds is not in their goitrogenic properties but rather in their interference with tests of thyroid function. This subject has recently been reviewed.[172]

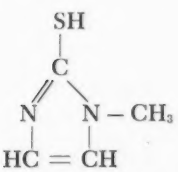
THIOCARBIMYL AND THIOCARBAMYL DERIVATIVES. Compounds in this category, originally classified by Astwood as containing the thioureylene radical, share the groupings



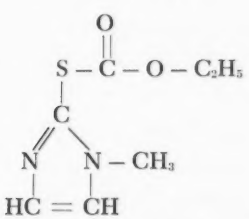
The largest number and the most potent antithyroid compounds thus far studied are included in this group. The following are currently the most important of those in clinical use:



6-*n*-Propyl-2-thiouracil
(propylthiouracil)



1-Methyl-2-mercaptoimidazole
(methimazole)



1-Methyl-2-carbethoxythioimidazole
(carbimazole)

The relative antithyroid activity obtained by assay of the inhibitory effect of thioureylene compounds on the uptake of radioactive iodine in man[111, 167] is as follows:

| | |
|---|---|
| Thiouracil | 1 |
| Methylthiouracil | 2 |
| Propylthiouracil | 0.75 |
| Methimazole | 100 |
| Carbimazole | 100 |

However, the potency of these compounds by this assay is not commensurate with their therapeutic effects. For example, methimazole is about 10 to 20 times as potent as propylthiouracil when estimated by its effect in the treatment of hyperthyroidism.[23, 84]

Members of this group of compounds containing polar substituents are highly ionized at pH 7, which might account for their low levels of antithyroid activity, since highly ionized organic compounds are in general poorly absorbed from the gastrointestinal tract.[149] Compounds that contain the thiocarbamyl or thiocarbimyl grouping will undoubtedly be synthesized in the future for use in the therapy of nonthyroidal systemic conditions; these should be studied to determine their effects on thyroid function tests.

MISCELLANEOUS COMPOUNDS. Classification of organic goitrogenic compounds has previously included only substances which contain either sulfur or nitrogen, or both, in the organic molecule. The studies of Arnott and Doniach[6] and of others[31, 54, 78] have demonstrated the goitrogenic properties of compounds related to resorcinol. Most of these contain two hydroxyl groups meta-oriented on the benzene ring, with the exception of 2,7-dihydroxy-naphthalene. Resorcinol derivatives and aniline compounds are inhibitors of peroxidase activity, and this has been postulated as their mechanism of action.[147] There are undoubtedly many substances not yet clearly classified which have antithyroid activity; clarification of their effects on thyroid function must await further investigation. The effects of pharmacologic substances on thy-

roid function have recently been reviewed by Grayson.[77]

***Pharmacologic properties of antithyroid drugs.*** There is little information concerning the absorption, excretion, distribution, and metabolism of the currently available antithyroid drugs. The most extensive studies on this subject have been carried out on thiouracil by Williams and associates.[186, 188, 189] Data obtained from these investigations may be summarized as follows: Approximately 80 per cent of a single oral dose of thiouracil disappears from the gastrointestinal tract within 2 hours, and 15 per cent of the drug is destroyed prior to absorption. The secretions of the stomach, duodenum, and jejunum, but not of the ileum, possess the capacity to inactivate these compounds. In man, thiouracil appears in the blood approximately 15 to 30 minutes after a single oral dose of 100 to 200 mg. After this, there is a gradual decline in the blood level with complete disappearance in approximately 48 to 72 hours. Effective blood levels can be fairly well maintained by the administration of small doses at frequent intervals. Thiouracil appears to circulate in the blood bound to the plasma proteins, from which it can be separated by ultrafiltration at low pH. It is estimated that concentration in cells is approximately 2 to 7 times that of plasma and that the erythrocyte content of thiouracil in a given specimen is roughly twice that of the leukocytes. Thiouracil seems to be distributed in all the tissues and fluids of the body, but at relatively different concentrations. Bone marrow contains greater quantities than any other tissue. The catabolism of thiouracil is more rapid than its derivatives substituted in the 6 position. Patients who receive more than 100 mg. of 6-*n*-propylthiouracil daily have blood concentrations greater than those achieved with the same dose of thiouracil. However, there is no difference in blood levels when the daily dosage is less than 100 mg. Concurrent administration of thyrotropin decreases the stores of thiouracil in the thyroid gland, while the administration of iodide appears to increase the concentration of the drug.

McGinty and colleagues[107] found that much less propylthiouracil was eliminated in the urine in 24 hours than thiouracil or methylthiouracil. Approximately 40 per cent of thiouracil or methylthiouracil is excreted in the urine in 24 hours; not more than 10 per cent of propylthiouracil is excreted as the free compound in the same period. After acid hydrolysis, 35 per cent of the drug is recovered.

Thus, propylthiouracil is conjugated in the body and is eliminated as an acid hydrolyzable complex of unknown nature. Conjugation of this drug could explain its relatively weaker activity. Thiouracil and methylthiouracil remain in the blood for longer periods and in slightly higher concentrations than propylthiouracil.

Recently, Spector and Sheridan[160] showed that thiouracil is transformed in vitro into its oxygen analogue. Since no thiourea could be demonstrated in experiments with thiouracil either in vivo or in vitro, it appears that primary cleavage of the thiopyrimidine ring with the formation of thiourea and acrylic acid does not occur. Therefore, thiouracil may follow a catabolic pathway such as thiouracil → uracil → β-uridoproprionic acid → β-alanine. This reaction is apparently accomplished by an enzyme requiring triphosphopyridine nucleotide and oxygen.

Effective blockade of the thyroid gland as measured by $I^{131}$ uptake is achieved rapidly and persists for several hours after the ingestion of these compounds. Adequate therapeutic effects are generally obtained with oral doses spaced at regular intervals of 6 to 8 hours. After discontinuance of the drug, there is progressive recovery of the gland and rapid return to either normal or increased function. In patients receiving antithyroid drugs, discontinuance leaves the thyroid gland in a hyperplastic, iodine-deficient state and under excessive thyrotropin stimulation. There is often a rebound effect manifested by elevated uptakes of radioactive iodine by the

gland. Thus, the radioactive iodine uptake test performed shortly after discontinuance of antithyroid drugs, even in normal individuals, may be in the range of hyperthyroidism and may not indicate the true metabolic status of the patient.

Antithyroid compounds readily transgress placental structures to affect the fetal thyroid gland.[69, 126] Relatively elevated concentrations are also found in the milk of lactating mothers.[186]

***Toxicity.*** Early investigations of antithyroid therapy revealed the rather pronounced toxicity of thiouracil and alerted physicians to use caution during clinical trials. Fortunately, most of the useful thiourea derivatives are much less toxic than thiouracil itself, and toxicity has not proved deterrent to their clinical usefulness.

According to a currently accepted classification of toxicity,[152] the unwanted reactions of drugs may be described as manifestations of overdosage, intolerance, untoward effects, secondary effects, idiosyncrasy, or hypersensitivity-allergic phenomena.

*Overdosage.* This problem with antithyroid drugs is generally not serious. These compounds may exert a toxic effect because of absolute overdosage, i.e., when the drug is injudiciously given in amounts larger than therapeutically necessary. Absolute overdose of antithyroid compounds may cause skin or bone marrow effects which disappear upon reduction of dosage. In euthyroid subjects, it is difficult to produce hypothyroidism even by excessively large doses because of the compensatory effects of endogenous thyrotropin. However, the problem of relative overdosage may become manifest in hyperthyroid patients treated with antithyroid drugs. The occurrence of hypothyroidism during the course of treatment does not necessarily depend upon the dose itself, for it may be related to the ease with which these compounds are capable of inhibiting the synthetic processes within a particular thyroid gland. Astwood[17] has pointed out that during maintenance therapy, as little as 25 mg. of propylthiouracil twice a day is sufficient to induce the development of myxedema in some patients, whereas withdrawal of treatment permits the return of hyperthyroidism.

*Intolerance.* The phenomenon of intolerance to the currently employed antithyroid drugs does not represent any real encumbrance to their effective use. Actual intolerance is probably quite uncommon and, when it does occur, is mild and readily controlled. Among its reported symptoms are gastrointestinal difficulties, hepatic disturbances, ophthalmic symptoms, and neurotoxic manifestations.[48] Large numbers of patients with thyrotoxicosis exhibit many identical manifestations without therapy. Jaundice and impaired hepatic function, nausea and vomiting, neuropsychiatric effects, musculoskeletal symptoms, cardiovascular alterations, skin lesions, lymphadenopathy, and a large group of miscellaneous signs and symptoms contribute to the protean clinical manifestations of hyperthyroidism. It is quite difficult at times to dissociate one phenomenon from the other. To what extent drug intolerance to antithyroid compounds modifies or contributes to these manifestations is difficult to assess.

*Untoward and secondary effects and idiosyncrasy.* Untoward effects and secondary effects have not been clearly described in the course of treatment of hyperthyroidism with antithyroid drugs. Also, true idiosyncrasy, implying inherent, qualitatively abnormal reaction, has not been noted.

*Hypersensitivity-allergic reactions.* By far the most important toxic reactions of antithyroid compounds are the sensitivity reactions. In 1948, the Council of Pharmacy and Chemistry of the American Medical Association[8, 44] reported it would sanction thiouracil only for preoperative treatment or for patients on whom operation was contraindicated, because of its marked toxic effects: urticarial, febrile, and agranulocytic reactions. Cookson and Staines[43] stated that most of the toxic effects with this drug were noted when the daily dose exceeded

0.1 Gm. The substantially lower toxicity of propylthiouracil may be attributed to its appreciably lower effective dosage. Patients sensitized to thiouracil can often be continued on therapy with full dosages of propylthiouracil[18, 76] or methylthiouracil[43] without necessarily showing recurrent toxicity. This is also true of mercaptoimidazole. With compounds of the imidazole group, toxic reactions generally increase rapidly in number when the dosage is raised above 50 mg. per day.[106]

In 1951, a derivative of methimazole was introduced for clinical use.[97, 98] This compound, 1-methyl-2-carbethoxythioimidazole (carbimazole), differs from methimazole by replacement of the hydrogen of the sulfhydryl group by a carbethoxy radical. The advantage of this derivative over the parent substance was its purportedly diminished catabolism with slow hydrolysis of the carbethoxy group to form the sulfhydryl compound. In this manner, a steady supply of the inhibiting compound would be available to the thyroid gland. In turn, smaller doses might possibly be required and thus appreciably reduce the incidence of toxic reactions. Kirkeby and Romcke[95] noted that the hydrolysis of 40 mg. of carbimazole yields only 25 mg. of methimazole. Fraser and associates,[67] on the basis of in vitro observations, were unable to support the conversion theory. The reported incidence of toxic reactions to carbimazole of 1.9 per cent[106, 176] appears to be somewhat lower than that attributed to other clinical drugs currently used. However, fatal bone marrow aplasia has been traced to this drug,[141] and more recent studies have shown it to have a higher incidence of toxicity.[46] The statement of Crooks and Wayne[46] is consistent with the experience of many investigators: "There is no convincing evidence that any of the organic antithyroid drugs is significantly more toxic than any other when it is given in therapeutically equivalent dosage."

Potassium perchlorate has only recently been used clinically as an antithyroid drug. Because of its simple inorganic nature, it would appear less likely to produce sensitization effects than the organic compounds, particularly on the hematopoietic system. Crooks and Wayne[46] reported that doses of 0.6 to 1 Gm. daily of this drug produced fewer toxic reactions than 1.5 to 2 Gm. Doses above 2 Gm. daily appeared to produce an increased incidence of skin rashes. They reported 1 case of agranulocytosis. Potassium perchlorate has not yet been submitted to sufficient clinical trial to evaluate fully its toxicity. Thus, the suggestion that it is the antithyroid drug of choice in the medical treatment of thyrotoxicosis is premature and cannot yet be supported by clinical experience.[46]

Drug reactions of the allergic-hypersensitivity variety may be grouped into three major categories: (1) the skin reactions, (2) granulocytopenia, agranulocytosis, and thrombocytopenia, and (3) a group of miscellaneous toxic reactions including drug fever, hepatitis, and others.

SKIN MANIFESTATIONS. All clinically employed antithyroid drugs appear to have about 3 to 5 per cent incidence of skin reactions. Fortunately, most of these are mild and often manifested by pruritic or maculopapular rashes or by urticaria. In many instances when the rash is mild, continuance of the drug under very careful observation and in conjunction with antihistaminic compounds will not result in any deleterious effects and the rash may disappear spontaneously. However, severe forms of dermatitis are indications for discontinuance of the inciting preparation. One must then employ either a compound with a different structural configuration or use some other form of therapy. Occasionally, bizarre skin manifestations, vascular purpura, or ectodermal changes in the hair or fingernails are seen which disappear with discontinuance of the drug. Many patients with untreated hyperthyroidism experience generalized pruritus, usually without any rash or at times associated with mild folliculitis; this is commonly a manifestation of the hyperthyroidism and should not be confused with a drug reaction.

GRANULOCYTOPENIA AND AGRANULOCYTOSIS. The approximate incidence of granulocytopenia is about 5 per cent in patients receiving the organic antithyroid drugs. The highest incidence occurs during the first few months of therapy. However, in untreated patients with hyperthyroidism, there is an associated granulocytopenia with a relative lymphocytosis and a slight reduction in total circulating white cell elements. Methimazole and propylthiouracil not uncommonly produce some further reduction in circulating granulocytes during the first several months of therapy. Usually this is not of any clinical significance, and the normal population of white cells returns gradually as euthyroidism is achieved. During the preliminary trials of therapy with these compounds, periodic white blood cell counts were commonly performed. These were tedious and expensive to the patient and were subsequently proved valueless in prognosticating bone marrow depression. Fortunately, serious bone marrow depressions from antithyroid therapy are relatively rare.

The term agranulocytosis should be reserved for the disorder characterized by the abrupt, fulminating privation of circulating granulocytes. This condition is quite different from granulocytopenia of gradual onset caused by a bone marrow depression[50] which appears to be dosage dependent. It is still not clear whether agranulocytosis resulting from sensitivity to antithyroid drugs is toxic or immunologic in nature. Although there is a paucity of supporting evidence in the literature[89] for the immunologic basis of this phenomenon caused by antithyroid drugs, it is an attractive hypothesis.

Clinically, it is particularly important to notify the physician at once if fever or severe sore throat occurs in patients on antithyroid drugs and to discontinue medication. A white blood cell and differential count is then required to determine the nature of the problem. In the rare patient in whom acute, severe bone marrow maturation arrest has occurred, immediate withdrawal of the drug, hospitalization with reverse precaution isolation, and judicious use of antibiotics, where indicated, appear to comprise the treatment of choice. Usually, steroids are necessary only if thrombocytopenia is also present. There were several deaths from agranulocytosis reported in the earlier literature.[176] More recently, because of greater appreciation of dosage, improved hematologic management, and more prompt recognition of this syndrome, severe granulocytopenia or even agranulocytosis has not proved to be a major deterrent to the use of these compounds. Most patients, if diagnosed and treated early, will experience complete recovery of the bone marrow in 1 week or 10 days, often with rebound leukocytosis. Moeschlin[121] has stated that if the bone marrow is still hyperactive with many myelocytes present, the prognosis is good provided the inciting cause is removed. If there are no myelocytes, or only a few, the situation is more severe, however recovery may still occur. If, in addition, there is joint swelling or jaundice, the prognosis is poor even with careful management employing full doses of antibiotics and cortisone.

Thrombocytopenic purpura has been reported in thyrotoxicosis both prior to[73] and after treatment with antithyroid drugs.[176] Regardless of cause, thrombocytopenic purpura is a grave complication in hyperthyroidism and must be vigorously treated. Bleeding diathesis from antithyroid drugs has also been reported. Craddock and associates[45] reported a severe case of coagulation defect in a patient under treatment with propylthiouracil. Administration of fresh, thrombin-free serum immediately improved the defect, presumed to be a deficiency of accelerator globulin. Miner[120] reported another case occurring in the absence of liver disease. D'Angelo and deGresley[49] presented a case in which propylthiouracil appeared to act as a coumarin-like anticoagulant; vitamin $K_1$ was successful in correcting the hypoprothrombinemia and hypoconvertinemia. A test

dose of propylthiouracil was readministered 9 days after discontinuance of therapy; a rapid fall in prothrombin and proconvertin (factor VII) was observed without any change in the other coagulation factors. The prothrombin time was easily brought back to normal with vitamin K. Greenstein[79] has reported still another case of hypoprothrombinemia in which propylthiouracil was the apparent cause of the depressed prothrombin time and in which vitamin K was also effective.

MISCELLANEOUS. There are a number of reported miscellaneous toxic manifestations attributed to antithyroid drugs. Notable among these is drug fever, which occurred in approximately 1 to 3 per cent of patients who received thiouracil. Fortunately, this is an uncommon reaction with methimazole or propylthiouracil. Hepatitis during the course of treatment with thiouracil,[70, 72, 88] propylthiouracil,[102] or methimazole,[153, 159] has been reported. Hepatic damage that occurs during treatment with thiouracil or methimazole resembles the type of intrahepatic biliary obstruction seen with chlorpromazine. Recovery after discontinuance of the drug generally occurs. The problem of drug-induced hepatic injury was recently reviewed by Popper and Schaffner.[134]

***Antithyroid drugs and tumorogenesis.*** The influence of antithyroid drugs on the genesis of thyroid tumors deserves brief comment. This subject was recently reviewed by Leathem.[99] Administration of a goitrogen and a carcinogen such as 2-acetylaminofluorene may speed the formation of adenomas, increase the frequency of nodules, and favor the development of carcinoma.[26] Radiation may act as a cocarcinogen[53] in the production of thyroid neoplasms. The subject of radiation in the induction of thyroid neoplasia has recently received widespread attention.[190]

Cellular alterations seen in the thyroid glands of patients under treatment with antithyroid drugs may be confused histologically with thyroid neoplasia. To our knowledge, there has been no documented case of carcinoma of the thyroid which can be attributed directly to the use of goitrogens in the treatment of hyperthyroidism.

***Effects of antithyroid drugs on radioactive iodine therapy.*** It has been known for some time that pretreatment of hyperthyroid subjects with antithyroid drugs alters the response to radioactive iodine therapy. Crooks and coauthors[47] concluded that pretreatment with methylthiouracil renders the gland relatively radioresistant. This radioprotective action of the drug might explain the results obtained by some investigators[66] who used larger doses of radiation. They reported a smaller incidence of hypothyroidism than those[30] who used smaller doses in patients not receiving pretreatment with antithyroid drugs.

### Therapeutic aspects of antithyroid drugs

A detailed discussion of the basis for selection of patients for antithyroid therapy, subtotal thyroidectomy, or radioactive iodine is beyond the scope of this review. The reader is referred to several comprehensive discussions of this important subject.[17, 40, 51, 93, 168, 181] However, a summary of currently accepted protocol is appropriate in order to establish the status of antithyroid medications among the several alternate forms of therapy.

***Childhood and adolescence.*** In general, children should be given an initial trial of long-term antithyroid therapy. This recommendation is based upon the observation that hyperthyroidism occurring at pubescence is frequently a mild disorder. Experience with antithyroid therapy in childhood and adolescence reveals a high rate of remission and cure.[185]

Van Wyk and colleagues[178] reviewed their 10 years' experience with antithyroid drugs in children and concluded on the basis of experience derived from study of 16 preadolescents that antithyroid drugs were the preferred form of therapy. Reduction in the size of the goiter during treatment was the best prognostic sign. It was suggested that the combined use of thyroid

with antithyroid drugs in those children with thyroidal enlargement might increase the number obtaining a permanent remission. They recommended that surgery be reserved for those who either were sensitive to antithyroid drugs or were unable to follow the proposed therapeutic regimen. A period of 2 years of continuous treatment was suggested as the minimum.

Hayles and associates[86] reviewed the experience of the Mayo Clinic in the surgical treatment of children from 1908 to 1955. They concluded that subtotal thyroidectomy is a satisfactory and safe procedure. There were 4 operative deaths, all prior to 1930. Twenty-eight of the 196 patients experienced recurrence of hyperthyroidism of such severity as to require further definitive treatment. Recurrences were not eliminated by more extensive resections but produced a higher percentage of hypothyroidism.

Arnold, Talbot, and Cope[5] analyzed the experience of the Massachusetts General Hospital during the 10 years prior to 1958 and compared the results of medical and surgical treatment. They concluded that both are equally capable of correcting the overt clinical manifestations of hyperthyroidism and that each yielded equal numbers of "cured" patients at the end of a 2 year period.

The serious consequences of hypoparathyroidism, myxedema, or other surgical complications are so particularly detrimental to the health and development of young people that subtotal thyroidectomy should be relegated to those few in whom adequate medical therapy has failed to effect a permanent cure. Since subtotal thyroidectomy can be performed at any stage in the medical treatment of this disorder, it should certainly not be given first preference. Radioactive iodine has no place in the therapy of hyperthyroidism in children and adolescents at the present time. This stems from current lack of knowledge of its genetic effects and from reports of an apparent increase in thyroid cancer after radiation of the neck in children.[190]

***Pregnancy.*** Antithyroid compounds are particularly indicated in pregnancy complicated by hyperthyroidism. Under these circumstances, subtotal thyroidectomy presents an unjustifiable hazard to the patient and the pregnancy and offers no advantages. Refinements in the use of antithyroid drugs and the avoidance of hypothyroidism by concomitant use of 2 to 3 grains USP thyroid permit a remarkable degree of success in the management of hyperthyroidism in pregnancy.[7, 7a, 15, 27, 64, 110] Radioiodine has no place in the treatment of hyperthyroidism in pregnancy because of potential hazards to the fetus and for genetic reasons. The subject of pregnancy and thyroid disease has been carefully reviewed.[24, 60, 68, 90]

***Adults under age 40.*** A great deal of controversy still persists concerning the most effective choice of therapy in this age group. Long-term management with antithyroid drugs has been successful in a relatively large group of young adults with small goiters and moderate degrees of hyperthyroidism in whom the size of the thyroid diminishes toward normal under treatment. However, only 60 to 70 per cent of all those receiving long-term programs are estimated to be cured by an adequate medical course; the remainder require either further prolonged antithyroid treatment or some form of thyroidal ablation. An increasing proportion of medical failures after one or more courses are currently being given radioactive iodine.[34, 63] However, final assessment of radioactive iodine therapy in this age group must wait 15 to 25 years in order for the effects of radiation on thyroid or bone marrow neoplasia to be evaluated fully. Until such time, it seems advisable to treat these patients by subtotal thyroidectomy. This subject has been extensively discussed in the literature with widely varying opinions.

Occasionally, short-term courses of antithyroid drugs are justified as a therapeutic trial if diagnostic measures are indecisive and clinical circumstances clearly indicate that other disorders which may simulate hyperthyroidism have been excluded.

***Radioactive iodine.*** The use of radioactive iodine, usually $I^{131}$, is rapidly increasing in many large medical centers. It is a convenient, easily administered, effective, and reliable form of ablation therapy which does not have the complications of surgical damage to recurrent laryngeal nerves or parathyroid glands. The incidence of hypothyroidism is about the same or perhaps slightly greater than after a surgical procedure, and the recurrence rate may be less. Unfortunately, the long-term effects of radiation are not now fully appreciated, either upon genetic factors or upon the induction of neoplasia. For this reason, most investigators have been cautious in the use of radiotherapy, especially in younger individuals. Radioactive iodine is usually employed in all patients beyond the childbearing age, arbitrarily set at 40 years or over. In addition, it is indicated in those in whom there is a medical contraindication to adequate elective operations or in whom the expected surgical risks outweigh the potential hazards of radiation. These include such concomitant disorders as severe diabetes, organic heart disease, ulcerative colitis, active tuberculosis, hepatic or renal insufficiency, and intractable psychiatric disorders. Although the anticipated fear of thyroid cancer caused by radioactive iodide therapy has not been borne out over the past 15 years, the incidence of leukemia after $I^{131}$ must be carefully surveyed to determine whether this is a result of radiation or a fortuitous event.[32, 157, 179] Radioiodide therapy is commonly recommended in the treatment of recurrent hyperthyroidism after subtotal thyroidectomy and occasionally as a therapeutic trial in cardiac patients in whom a diagnosis of hyperthyroidism cannot be clearly established.[150] Comprehensive reviews of the treatment of hyperthyroidism with radioactive iodine have recently appeared.[30, 34, 37, 56, 63]

***Selection of antithyroid compounds.*** In the United States two antithyroid drugs are now almost exclusively employed in the treatment of hyperthyroidism. These are methimazole* and propylthiouracil. In Great Britain, a derivative of methimazole called carbimazole† is popularly employed.

There is essentially no fixed guide for the selection of one of these compounds in favor of the other. Methimazole has about 10 to 20 times the therapeutic effectiveness in man of propylthiouracil. When administered in the usual therapeutic doses of 10 mg. every 8 hours, it has a more rapid action and a somewhat greater incidence of toxic effect than propylthiouracil (100 mg. every 8 hours). This difference in toxicity between the two compounds appears to be a result of the relatively larger doses of methimazole employed, since 5 mg. every 8 hours gives essentially the same therapeutic response as 100 mg. of propylthiouracil given similarly.[23] Thus, these compounds are probably equally effective when administered in these latter doses and have a comparable incidence of toxic manifestations. The advantage of having several preparations available is related to their chemical dissimilarity—one may be used in place of the other when minor toxic reactions occur, especially skin manifestations. Obviously, serious toxic effects on the bone marrow do not permit changing to any compound which may have similar effects and usually indicate an ablative form of therapy. The iodinated derivative of thiouracil, 5-iodo-2-thiouracil, has not exhibited the therapeutic advantages originally conceived in a compound combining a goitrogenic drug with iodide.[148, 175] Currently it is employed only occasionally.

A derivative of methimazole, carbimazole, was introduced for clinical trial in 1951. Since then, it has been tested fairly extensively in both Great Britain and the United States.[22, 46, 52, 105, 106, 133] It does not have any particular advantages over methimazole or any significant decrease in side effects.

*Tarpazole, Mercazole.

†NeoMercazole.

Potassium perchlorate, although not yet accepted as an official antithyroid drug, is gaining therapeutic importance. It has the advantages of being inexpensive and of being equally effective as the organic compounds, and it rarely has any untoward effects on the bone marrow.[46] Unlike other antithyroid drugs, it may cause gastric irritation if not taken with meals. At present, it is used in individuals in whom toxic reactions to the organic antithyroid compounds have occurred and in whom it is desirous to continue drug therapy.

The most important factor in the selection of any antithyroid drug is the familiarity of the physician with the desired and undesired properties of that particular compound.

***Management of patients receiving antithyroid therapy.*** Certain general principles pertaining to the use of antithyroid drugs have evolved over the past 17 years. In patients with diffusely enlarged glands, the usual initial dosage of propylthiouracil is 300 to 400 mg. and of methimazole 20 to 30 mg. given daily in three equally spaced doses. This regimen will accomplish euthyroidism in well over 90 per cent of these patients. When an inadequate response to this dosage of either compound is noted after 4 to 6 weeks of therapy, failure to take the drug as directed or at the prescribed intervals is usually the cause. Patients with large or nodular goiters may occasionally require proportionately increased doses of these medications (even up to 5 times the usual daily doses) for accomplishment of euthyroidism. Potassium perchlorate shares with the organic antithyroid drugs a reasonable amount of allergic-sensitivity reactions, especially skin rashes and urticaria, but appears to have fewer effects on the bone marrow. Its starting dose should be at least 1 Gm. daily in three or four divided doses. A convenient and effective schedule is to administer 250 mg. by mouth four times daily in equally spaced amounts.

The initial dosage schedule of antithyroid compounds should be designed to produce relatively complete inhibition of thyroid hormonogenesis. If this is accomplished, most patients experience some improvement in symptoms approximately 2 to 3 weeks after the onset of therapy, and objective changes are often demonstrable after about 3 to 4 weeks. Once euthyroidism has been achieved, continuance of these doses may produce hypothyroidism in a small but significant number of patients. In order to obviate this, most investigators have recommended that the dose be reduced progressively until the minimal inhibitory amount which will maintain euthyroidism is determined. This particular method of arriving at a maintenance dose has several disadvantages: (1) the patient must be seen at frequent intervals; (2) because of the undulating nature of hyperthyroidism, patients who are euthyroid on one particular dose may subsequently be slightly hyperthyroid or hypothyroid on that same dose, and (3) physiologically, it seems unwise to maintain the inhibition of the thyroid gland at a threshold level at which it is constantly under stimulation to overcome the block and produce hormone. For these reasons, in 1957 Fraser[65] suggested that L-thyroxine be administered together with the antithyroid drugs so that full blocking doses could be continued for prolonged periods without the fear of inducing hypothyroidism.

For several years we have routinely added daily doses of 2 grains of USP thyroid or 100 $\mu$g L-triiodothyronine (liothyronine) to the therapeutic program of all patients receiving antithyroid therapy; thyroid replacement therapy is initiated at the time euthyroidism is first achieved. Thus, the patient can be conveniently maintained on relatively large doses of an antithyroid compound for 8 to 12 months in order to block as completely as possible the synthesis of thyroid hormones. The advantages of such a program are: (1) it obviates the occurrence of hypothyroidism, (2) it appears to have a beneficial effect on reducing goitrogenesis, (3) it seems to be effective in controlling the milder forms of

ophthalmopathy and perhaps in modifying the degree of exophthalmos (if present), (4) it obviates the necessity for frequent patient visits to modify the dosage schedule, and (5) physiologically, it seems wisest to put the thyroid gland at complete rest and at the same time avoid hypothyroidism. This therapeutic program has an additional advantage as far as prognostication is concerned. Once the patient has completed 8 to 12 months of therapy, the dosage of antithyroid compounds is progressively decreased at 3 month intervals until discontinued. However, the thyroid medication is simultaneously continued in the same dose. Three months after discontinuance of the antithyroid compound, a radioactive iodine uptake test is performed while the patient is still receiving the thyroid medication. If this uptake is low, it is a good prognostic sign that physiologic regulation of the patient's thyroid-pituitary mechanism has returned toward normal and a recurrence of antithyroid therapy is unlikely. On the other hand, if the $I^{131}$ uptake is elevated while the patient is taking suppressive doses of thyroid hormones, this indicates that a recurrence is likely since the thyroid gland is still incapable of suppression.[35] In these latter individuals, one is apt to suggest either radioactive iodine or subtotal thyroidectomy as an alternate form of therapy.

The concomitant use of Lugol's solution or some iodine preparation has been recommended as an adjunctive measure to antithyroid therapy. The experimental basis for this is that in animals receiving goitrogens alone, there is marked glandular hyperplasia. The addition of iodide causes involution of the gland with accumulation of colloid deficient in thyroid hormones. However, from a clinical standpoint, the administration of iodide has not increased the rapidity with which euthyroidism is achieved, neither has it added any beneficial effects to the treatment of patients. It has the decided disadvantage of rendering inaccurate the accomplishment of a protein-bound iodine determination which is frequently necessary in following these individuals.

***Preparation of hyperthyroid subjects for ablative therapy.*** The effective management of hyperthyroid patients with antithyroid drugs in anticipation of subtotal thyroidectomy is worthy of some comment here. Generally, it is advisable to treat such patients from 4 months to 1 year prior to elective thyroidectomy. During this period, they are able to recover fully from the metabolic effects of the disorder and to be restored to physiologic homeostasis.[7, 8] Furthermore, if ophthalmopathy exists, a generously prolonged period of medical treatment is desirable to stabilize this condition and to avoid the exacerbations of exophthalmos sometimes seen when surgical operation is performed after the rapid accomplishment of euthyroidism. Subtotal thyroidectomy should be an *elective* procedure performed only under optimally beneficial circumstances.

Iodide is required in the preoperative preparation of hyperthyroid patients who are receiving antithyroid therapy. In order to reduce the vascularity and friability of the hyperplastic thyroid gland, Lugol's solution or saturated solution of potassium iodide is added to the antithyroid regimen for 10 days prior to surgical operation. In patients treated with potassium perchlorate, administration of iodide will overcome the perchlorate block and produce a mild form of "Jod Basedow" (hyperthyroidism caused by the administration of iodide[74]). Therefore, in order to prepare for surgical procedure a patient who has been receiving potassium perchlorate, an organic goitrogen should be substituted for the perchlorate in the immediate preoperative period, during which time iodide can be successfully administered.

The use of radioactive iodine is currently resulting in referral of a substantial number of patients in this age group for radiothyroidectomy. In many large clinics, increasing numbers of patients between the ages of 30 and 40 are being treated with $I^{131}$ therapy. The wisdom or folly of using

radiation therapy in young adults must await prolonged observation for the consequences of radiation on neoplasia or genetics.

Use of antithyroid drugs as pretreatment in patients selected for radioactive iodine therapy is usually not necessary. Radiation thyroiditis, even in thyrocardiac patients, has rarely occurred with sufficient severity to necessitate antithyroid therapy prior to use of $I^{131}$. Pretreatment with antithyroid compounds may necessitate use of larger doses of radioactive iodine than in untreated patients.[47]

***Management of hyperthyroidism in pregnancy.*** Hyperthyroidism complicating pregnancy represents one of the most important indications for the use of antithyroid treatment. As previously noted during the course of an uncomplicated pregnancy,[33] the added oxidative burdens of gestation produce progressive increments in the basal metabolic rate, so that by the third trimester, it has achieved levels of +25 to +35 per cent of the normal standard. For this reason, no effort should be made to lower the basal metabolic rate below these levels. Reports of congenital goiters, cretinism and fetal deaths are now explained as the results of injudicial overdosage because of insufficient appreciation of this fact.[57, 148] The administration of 2 to 3 grains of USP thyroid or 100 $\mu$g liothyronine along with the antithyroid drug obviates the need for concern of hypothyroidism either in mother or in fetus. This was originally suggested by Astwood[15] and has subsequently been employed by others,[7a] although not so didactically stated in the literature.

On such a regimen, no significant goiters or untoward fetal consequences should be noted in the newborn infants. The dosage of antithyroid compound should be kept somewhat lower than in nonpregnant individuals; usually daily doses in the range of 200 mg. of propylthiouracil during the first trimester, 150 mg. during the second, and approximately 100 mg. during the third are quite adequate for control of the disorder, which is often mild in pregnancy. Subtotal thyroidectomy in this period presents hazards which are not justified if antithyroid drugs are properly employed.

***Management of complications of antithyroid therapy.***

*Toxic reactions.* By far the most common toxic manifestations of antithyroid compounds are skin rashes or urticarial reactions. These are usually not serious and may disappear with reduction in dosage. However, if generalized, it is usually wisest to discontinue the offending compound and to substitute cautiously one of dissimilar chemical structure, carefully watching for any recurrence. If the skin reaction is severe or if it recurs after the substitution of another agent, an alternate form of therapy is indicated. Manifestations of bone marrow toxicity may not be treated so lightly. Maturation arrest or marrow aplasia, although uncommon with the currently employed antithyroid compounds, is a serious and potentially fatal complication. Acute bone marrow "anaphylaxis" is quite rare, but its potentiality must always be apparent. Patients must be cautioned to report fever or sore throat immediately and to discontinue medication until its origin has been determined. Depression of the total leukocyte count is common, especially with larger doses of these compounds; this usually responds to reduction in dosage, and therapy need not be discontinued. If the total quantity of circulating polymorphonuclear leukocytes falls below 1,500, the drug should be discontinued. Potassium perchlorate, which appears to have the least bone marrow toxicity, may be substituted; the patient should then be observed carefully. When the granulocytopenia is severe, alternate forms of therapy such as radioactive iodine or subtotal thyroidectomy are advisable.

*Therapeutic failures.* It is extremely rare for a hyperthyroid patient with a diffuse goiter not to respond to adequate doses of antithyroid drugs if they are properly administered. In those who are unable to follow a prescribed program or who do not respond well to medical treatment, alter-

nate forms of therapy with radioactive iodine or subtotal thyroidectomy should be considered. In the final analysis, selection of the most suitable therapy must be tailored to the needs of the patient.

*Recurrent hyperthyroidism.* Recurrence of hyperthyroidism after a well-conceived and well-directed long-term antithyroid program is usually an indication for ablative therapy. When recurrence has followed a course of 2 years or more, subtotal thyroidectomy is usually advisable in younger individuals and radioactive iodine almost exclusively in the remainder. Recurrence of hyperthyroidism after subtotal thyroidectomy is a clear indication for the use of radioactive iodine.

*Thyrotoxic ophthalmopathy.* Severe and progressive exophthalmos does not occur with the same frequency after the judicious use of antithyroid compounds as after ablative procedures. It is therefore desirable to use medical therapy initially in patients with severe ophthalmopathy or progressive exophthalmos. This permits a period of observation to determine whether stabilization of the ophthalmopathy has occurred and to assess any progression of the disorder. In individuals with ophthalmopathy, it is particularly important to employ 2 to 3 grains of USP thyroid or 100 to 150 $\mu$g liothyronine concomitantly with medical therapy. Ablative procedures should be avoided in patients with severe exophthalmos with evident progression of the proptosis.

***Results of antithyroid therapy.*** It is particularly difficult to assess objectively a form of medical therapy such as the treatment of hyperthyroidism with antithyroid drugs. Reports of the effectiveness of various compounds from several different clinics are often not comparable because of differences in dosage, length of therapy, and adjunctive measures employed. Bias is often responsible for preferential selection of patients. Thus, over-all results of long-term antithyroid therapy must be judged in terms of a range of response rather than by a specific percentage. One should refer to "remissions" of some fixed duration with antithyroid compounds rather than "cures."

It is well known that untreated hyperthyroidism may follow an undulating course with intervals of spontaneous remission and exacerbation. Some patients seem to do extremely well with only short courses of therapy, whereas others seem to be refractive to all forms of therapy including subtotal thyroidectomy or radioactive iodine. Indeed, as Astwood[17] has recently pointed out, it is difficult to understand how any of the currently employed therapeutic measures bring about a cure. Presumably, antithyroid medications block the production of excessive quantities of thyroid hormones while whatever underlying abnormality responsible for the disorder has an opportunity to correct itself. Even more difficult to appreciate is the manner by which ablative procedures correct the abnormality. Removal of part of the thyroid gland quantitatively limits the hormonal production. But unlike normal thyroid tissue, the hyperthyroid gland usually neither regenerates nor atrophies after subtotal thyroidectomy. This static condition of the gland after clinical correction of the disorder is even more puzzling since the remnant may often remain hyperactive or retain the defect of failure of suppression.[183] Perhaps the most reasonable explanation is that the currently employed measures are not curative but provide a period of control of the disorder during which time the patient may experience spontaneous recovery.

*Long-term therapy.* Most of the available information concerning results of antithyroid therapy was collected and critically reviewed in 1955 by Vanderlaan and Storrie.[176] Subsequent series have, for the most part, been directed toward comparative studies of the newer compounds, carbimazole and potassium perchlorate. At present, certain generalizations concerning results of therapy are permissible:

1. A decrease in the size of the thyroid gland during therapy is generally a good

prognostic sign.[158, 178, 187, 191] Such other factors as age, sex, goiter size, nodularity, duration of illness, severity, previous treatment, and duration of treatment, while important, have not been definitive in the assessment of therapeutic results.[158] However, small goiters appear more amenable to therapy than large or nodular ones.[91, 132, 191]

2. Most investigators regard therapy of less than 1 year's duration as inadequate.[23, 33, 187, 191] Although unsupported by critical evidence, treatment for longer periods seems to be associated with more favorable results.

3. The percentage of permanent remissions following discontinuance of therapy remains a controversial subject. Results reported by various investigators are biased by manner of selection and by limited follow-up periods. The marked divergence of results was reviewed by Bishop[28] who noted reports varying from relapse in "nearly every case"[169] to 90 per cent remission rates.[118] However, most series suggest an over-all remission rate of about 50 to 60 per cent. McCullagh and Cassidy[103] in a study 4 to 6 years after discontinuance of therapy on propylthiouracil, noted remissions in 66.7 per cent. The report of Solomon and co-workers[158] with unselected patients showed a remission rate of 55 per cent after one course of treatment and 70 per cent after more than one course.

4. Progressive or malignant exophthalmos appears to be less aggravated by antithyroid drugs than by ablative forms of treatment.[28, 191]

Astwood[17] has recently expressed some generalizations on treatment with antithyroid drugs from his very long and critical experience. He states that the relapse rate may be at least 50 per cent for patients receiving a single course of treatment with antithyroid drugs and that even after multiple courses, one-fourth to one-third repeatedly relapsed. Thus, there may be as much as a 25 to 35 per cent relapse rate. Because of this, individuals in the group between 20 and 40 years of age may eventually require subtotal thyroidectomy or radioactive iodide. The former should be avoided, if possible, in younger individuals because of the hazard of damage to the laryngeal nerve or the induction of hypoparathyroidism or myxedema. The advantages of radioactive iodine therapy in older individuals currently outweigh any theoretical deterrents, especially if there are cardiovascular complications or associated heart disease.

Finally, selection of the most appropriate form of therapy must be highly individualized. The ultimate choice not only is dependent upon highly problematical arguments as to the most expedient form of therapy, but is related to social, psychologic, and economic factors which often weigh heavily in the decision. Despite all attempts at objectivity, there still remain long-standing prejudices in favor of one form of therapy or another. When there is a valid choice, there can certainly be no objection to an initial trial of antithyroid therapy. If this fails or if other indications suggest that the treatment will not eventually be beneficial, certainly either subtotal thyroidectomy or radioactive iodine may be elected.

### *References*

1. Albert, A., Rawson, R. W., Merril, P., Lennon, B., and Riddell, C.: Reversible inactivation of thyrotropic hormone by elemental iodine. I. The action of iodine, J. Biol. Chem. **166**:637, 1946.
2. Anbar, M., Guttman, S., and Lewitus, Z.: Effect of monofluorosulphonate, difluorophosphate and fluoroborate ions on the iodine uptake of the thyroid gland, Nature, London **183**:1517, 1959.
3. Anderson, G. W.: Antithyroid compounds, *in* Burger, A., editor: Medicinal chemistry, vol. I, New York, 1951, John Wiley & Sons, Inc.
4. Andrews, G. A.: A few notions involved in the clinical use of radioisotopes, Ann. Int. Med. **47**:922, 1957.
5. Arnold, M. B., Talbot, N. B., and Cope, O.: Concerning the choice of therapy for childhood hyperthyroidism, Pediatrics **21**:47, 1958.
6. Arnott, D. G., and Doniach, I.: The effect of compounds allied to resorcinol upon the uptake of radioactive iodine ($^{131}I$) by the thyroid of the rat, Biochem. J. **50**:473-479, 1952.

7. Asper, S. P.: Observations on the preparation of hyperthyroid patients for subtotal thyroidectomy with propylthiouracil, Surgery **34**: 655, 1953.
7a. Asper, S. P., and London, F.: Thyrotoxicosis and pregnancy, 1961. In press.
8. Asper, S. P.: The use of the antithyroid drugs in the treatment of hyperthyroidism, Practitioner **173**:611, 1954.
9. Astwood, E. B.: The chemical nature of compounds which inhibit the function of the thyroid gland, J. Pharmacol. & Exper. Therap. **78**:79, 1943.
10. Astwood, E. B.: Treatment of hyperthyroidism with thiourea and thiouracil, J.A.M.A. **122**: 78, 1943.
11. Astwood, E. B.: Thiouracil treatment in hyperthyroidism, J. Clin. Endocrinol. **4**:229, 1944.
12. Astwood, E. B.: Chemotherapy of hyperthyroidism, Harvey Lect. **40**:195-235, 1945.
13. Astwood, E. B.: Further studies on the chemical nature of compounds which inhibit the function of the thyroid gland, Endocrinology **37**:456, 1945.
14. Astwood, E. B.: Mechanism of action of various antithyroid compounds, Ann. New York Acad. Sc. **50**:419-443, 1949.
15. Astwood, E. B.: The use of antithyroid drugs during pregnancy, J. Clin. Endocrinol. **11**: 1045, 1951.
16. Astwood, E. B.: Mechanism of action of antithyroid compounds, *in* The thyroid, Brookhaven Symposium in Biology, 1955, No. 7.
17. Astwood, E. B.: Treatment of hyperthyroidism, *in* Clinical Endocrinology, vol. I, New York, 1960, Grune & Stratton, Inc.
18. Astwood, E. B., and Vanderlaan, W. P.: Treatment of hyperthyroidism with propylthiouracil, Ann. Int. Med. **25**:813, 1946.
19. Astwood, E. B., Greer, M. A., and Ettlinger, M. G.: 1-5-Vinyl-2-thiooxazolidone, an antithyroid compound from yellow turnip and Brassica seeds, J. Biol. Chem. **181**:121, 1949.
20. Balint, J. H., Fraser, R., and Hanno, M. G. W.: Radioiodine measurements of thyroid function during and after PAS treatment of tuberculosis, Brit. M. J. **1**:115, 1954.
21. Barker, M. H.: The blood cyanates in the treatment of hypertension, J.A.M.A. **106**: 762, 1936.
22. Bartels, E. C.: Clinical experience with a new anti-thyroid drug: 2-carbethoxythio-1-methylglyosaline, J. Clin. Endocrinol. **13**:1305, 1953.
23. Bartels, E. C., and Sjogren, R. W.: 1-Methyl-2-mercaptoimidazole, a new active antithyroid agent, J. Clin. Endocrinol. **11**:1057, 1951.
24. Bell, G. O., and Hall, J.: Hyperthyroidism and pregnancy, M. Clin. North America **44**: 303, 1960.
25. Berson, S. A.: Pathways of iodine metabolism, Am. J. Med. **20**:653-669, 1956.
26. Bielschowsky, F.: Tumours of the thyroid produced by 2-acetyl-aminofluorene and allylthiourea, Brit. J. Exper. Path. **25**:90, 1944.
27. Billins, F. T., Jr., and Towery, B. T.: Thyrotoxicosis and pregnancy, Am. Practit. & Digest Treat. **8**:1052, 1957.
28. Bishop, P. M. F.: The management of thyrotoxicosis, *in* Recent advances in endocrinology, New York, 1954, Blakiston Company.
29. Blizzard, R.: Inherited defects of thyroid hormone synthesis and metabolism, Metabolism **9**:232, 1960.
30. Blomfield, G. W.: Treatment of thyrotoxicosis with $I^{131}$; review of 500 cases, Brit. M. J. **1**:63, 1959.
31. Bull, G. M., and Fraser, R.: Myxedema from resorcinol ointment applied to leg ulcers, Lancet **1**:851, 1950.
32. Burns, T. W., Vickers, R., and Lowney, J. F.: Acute leukemia after radioactive iodine ($I^{131}$) therapy for hyperthyroidism, Arch. Int. Med. **106**:97, 1960.
33. Burwell, C. S., and Metcalfe, J.: Heart disease and pregnancy, Boston, 1958, Little, Brown & Company, p. 27.
34. Cassidy, C. E., and Astwood, E. B.: Evaluation of radioactive iodine ($I^{131}$) as treatment for hyperthyroidism, New England J. Med. **201**:53, 1959.
35. Cassidy, C. E., and Vanderlaan, W. P.: Thyroid-suppression test in the prognosis of hyperthyroidism treated by antithyroid drugs, New England J. Med. **262**:1228, 1960.
36. Chaikoff, I. L., and Taurog, A.: Studies on the formation of organically-bound iodine compounds in the thyroid gland in their appearance in plasma as shown by the use of radioactive iodine, Ann. New York Acad. Sc. **50**:377, 1949.
37. Chapman, E. M., and Maloof, F.: The use of radioactive iodine in the diagnosis and treatment of hyperthyroidism: Ten years' experience, Medicine **34**:261, 1955.
38. Chesney, A. M., Clawson, T. A., and Webster, B.: Endemic goiter in rabbits, Bull. Johns Hopkins Hosp. **43**:261, 1928.
39. Choufoer, J. C., Kassenaar, A. A. H., and Querido, A.: The syndrome of congenital hypothyroidism with defective dehalogenation of iodotyrosines, J. Clin. Endocrinol. **20**:983, 1960.
40. Clark, D., and Rule, J. H.: Radioactive iodine or surgery in treatment of hyperthyroidism, J.A.M.A. **159**:995, 1955.
41. Clayton, G. W., Smith, J. D., and Leiser, A.: Familial goiter with defect in intrinsic metabolism of thyroxin without hypothyroidism, J. Pediat. **52**:129, 1958.

42. Clements, F. W.: Naturally occurring goitrogens, Brit. M. Bull. **16**:133, 1960.
43. Cookson, H., and Staines, F. H.: Thiouracil in toxic goitre, Brit. M. J. **2**:759-762, 1947.
44. Council on Pharmacy and Chemistry, J.A.M.A. **136**:691, 1948.
45. Craddock, C. G., Jr., Shotten, D., Crockett, C. L., Jr., and Leavell, B. G.: Severe hypoprothrombinemia following propylthiouracil treatment of thyrotoxicosis, New England J. Med. **244**:549, 1951.
46. Crooks, J., and Wayne, E. J.: A comparison of potassium perchlorate, methylthiouracil, and carbimazole in the treatment of thyrotoxicosis, Lancet **1**:401, 1960.
47. Crooks, J., Buchanan, W. W., Wayne, E. J., and MacDonald, E.: Effect of pretreatment with methylthiouracil on results of $I^{131}$ therapy, Brit. M. J. **1**:151, 1960.
48. Curtis, G. M., and Swenson, R. E.: Thiouracil in treatment of hyperthyroidism, Internat. Abstr. Surg. **86**:105, 1948.
49. D'Angelo, G., and deGresley, L. P.: Severe hypoprothrombinaemia after propylthiouracil therapy, Canad. M. A. J. **81**:479, 1959.
50. Discombes, G.: *in* The discussion of agranulocytosis due to sensitivity to drugs. Sensitivity reactions to drugs, Oxford, 1958, Blackwell Scientific Publications, p. 389.
51. Discussion of present-day views on treatment of thyroid conditions (other than cancer), Proc. Roy. Soc. Med. **52**:167, 1959.
52. Doniach, D.: Treatment of thyrotoxicosis with "NeoMercazole" (2-carbethoxy-thio-1-methylglyoxaline), Lancet **1**:873, 1953.
53. Doniach, I., quoted by Leathem, J. H.: Brit. J. Cancer **7**:181, 1953.
54. Doniach, I., and Fraser, R.: Effect of resorcinol on the thyroid uptake of $I^{131}$ in rats, Lancet **1**:855, 1950.
55. Einhorn, J., and Larsson, L. G.: Studies on effect of thyrotropin on human thyroid function, J. Clin. Endocrinol. **19**:28, 1959.
56. Eller, M., Silver, S., Yohalem, S. B., and Segal, R. L.: The treatment of toxic nodular goiter with radioactive iodine, Ann. Int. Med. **52**:976, 1960.
57. Elphinstone, N.: Thiouracil in pregnancy; its effect on the foetus, Lancet **1**:1281, 1953.
58. Endemic goiter, Monograph in Medicine and Public Health No. 12, Cambridge, Mass., 1954, Harvard University Press.
59. Endemic goiter, Geneva, 1960, World Health Organization.
60. Engstrom, W. W.: Thyroid function in pregnancy, Postgrad. Med. **27**:180, 1960.
61. Ettlinger, M. G., and Lundeen, A. J.: The structures of sinigrin and sinalbin; an enzymatic rearrangement, J. Am. Chem. Soc. **78**:4172-4173, 1956.
62. Federman, D., Robbins, J., and Rall, J. E.: Some observations on cretinism and its treatment, New England J. Med. **259**:610, 1958.
63. Feitelberg, S., Kaunitz, P. S., Silver, S., Simon, N., Wasserman, L. R., and Yohalem, S. B.: Hyperthyroidism; treatment with radioactive iodine, Arch. Int. Med. **85**:471, 1950.
64. Fraser, T. R., and Fisher, H. J.: Anti-thyroid drugs in pregnancy, Lancet **2**:89-90, 1953.
65. Fraser, T. R., and Wilkinson, M.: Simplified method of drug treatment for thyrotoxicosis, Brit. M. J. **1**:481, 1953.
66. Fraser, T. R., Abbatt, J. D., and Stewart, F. S.: Radioiodine treatment of thyrotoxicosis: A single dose method following a drug preparation, Brit. J. Radiol. **27**:23, 1954.
67. Fraser, T. R., Garrod, O., Hanno, M. G. W., and Jadresic, A.: Antithyroid activity and toxicity of mercazole and neo-mercazole, J. Clin. Endocrinol. **14**:1230-1244, 1954.
68. Freedberg, I. M., Hamolsky, M. W., and Freedberg, A. S.: The thyroid gland in pregnancy, New England J. Med. **256**:505, 551, 1957.
69. Freiesleben, E., and Kjerulf-Jensen, K.: The effect of thiouracil derivatives on fetuses and infants, J. Clin. Endocrinol. **7**:47, 1947.
70. Gabrilove, J. L., and Kert, M. J.: Sensitivity to thiouracil, J.A.M.A. **124**:504, 1944.
71. Gardner, J. U., Hayles, A. B., Woolner, L. B., and Owen, C. A., Jr.: Iodine metabolism in goitrous cretins, J. Clin. Endocrinol. **19**:638, 1959.
72. Gargill, S. L., and Lesses, M. F.: Toxic reactions to thiouracil, J.A.M.A. **127**:890, 1945.
73. Girsh, L. S., and Myerson, R. M.: Thyrotoxicosis associated with thrombocytopenia and hypersplenism, Am. J. Clin. Path. **27**:328, 1957.
74. Godly, A. F., and Stanbury, J. B.: Preliminary experience in the treatment of hyperthyroidism with potassium perchlorate, J. Clin. Endocrinol. **14**:70, 1954.
75. Goldsmith, R., Herbert, C., and Lutsch, G.: The effect of iodide on the release of thyroid hormone in hyperthyroidism; further observations, J. Clin. Endocrinol. **18**:367, 1958.
76. Grauer, R. C., DeWalt, H., and Elkin, C. W. W.: Thiouracil and propylthiouracil in the pre-surgical and medical management of thyrotoxicosis, Am. J. M. Sc. **215**:63, 1948.
77. Grayson, R. R.: Factors which influence the radioactive iodine thyroidal uptake test, Am. J. Med. **28**:397, 1960.
78. Greene, R., Farran, H., and Glascock, R. F.: Goitrogens in milk, J. Endocrinol. **17**:272, 1958.
79. Greenstein, R. H.: Hypoprothrombinemia due to propylthiouracil therapy, J.A.M.A. **173**: 1014, 1960.

80. Greer, M. A.: Isolation from rutabaga seed of progoitrin, the precursor of naturally occurring antithyroid compounds, goitrin (1-5-vinyl-2-thiooxazolidone), J. Am. Chem. Soc. **78**:1260, 1956.
81. Greer, M. A., and DeGroot, L. J.: A study of the effect of stable iodide on the thyroid secretion in man, Metabolism **5**:682, 1956.
82. Griesbach, W. E., Kennedy, T. H., and Purves, H. D.: Studies on experimental goitre. III. The effect of goitrogenic diet on hypophysectomized rats, Brit. J. Exper. Path. **22**:249-254, 1941.
83. Gualco, S., and Patrono, V.: Sugli effetti clinici e metabolici de tiouracile, del metiltiouracile, dell'aminotiazolo e dell acido paraaminobenzoico, Minerva med. **2**:353, 1947.
84. Hallman, B. L., and Bondy, P. K.: Experience with methimazole (Tapazole) in the treatment of hyperthyroidism, Am. J. Med. **11**:724, 1951.
85. Hamolsky, M. H., and Freedberg, A. S.: The thyroid gland, New England J. Med. **262**:23-28, 70-78, 129-137, 1960.
86. Hayles, A. B., Kennedy, R. L. J., Beahrs, O. H., and Woolner, L. B.: Exophthalmic goiter in children, J. Clin. Endocrinol. **19**:138, 1959.
87. Hercus, C. E., and Purves, H. D.: Studies on endemic and experimental goitre, J. Hyg. **36**:182-203, 1936.
88. Holoubek, J. E., Mathews, W. R., and Hollis, W. J.: Thiouracil hepatitis, Am. J. Med. **5**: 138, 1948.
89. Huguenin, A., Albou, A., and Griguer, P.: Agranulocytose par methylthiouracile avec hétéro-agglutinines antileucocytaires, Sang **30**: 207, 1959.
90. Osario, C., and Myant, N. B.: The passage of thyroid hormone from mother to foetus and its relation to foetal development, Brit. M. Bull. **16**:159, 1960.
91. Iversen, K.: Late results from continuous treatment of thyrotoxicosis with methylthiouracil, J. Clin. Endocrinol. **11**:298, 1951.
92. Jaimet, C. H., and Thode, H. G.: Thyroid function studies on children receiving cobalt therapy, J.A.M.A. **158**:1353-1355, 1955.
93. Keating, F. R., Jr., Patterson, H. A., and Vanderlaan, W. P.: Radioactive iodine, surgery or antithyroid drugs in treatment of Graves' disease, Postgrad. Med. **23**:78, 1958.
94. Kennedy, T. H., and Purves, H. D.: Studies on experimental goiter. I. The effect of Brassica seed diets on rats, Brit. J. Exper. Path. **22**:241-244, 1941.
95. Kirkeby, K., and Romcke, O.: Carbimazole in thyrotoxicosis, Lancet **1**:374, 1955.
96. Laidlaw, J. C.: Nature of circulating thyroid hormone, Nature, London **164**:927, 1949.
97. Lawson, A., and Barry, G.: Treatment of thyrotoxicosis with 2-carbethoxy-thio-1-methyl-glyoxaline, Lancet **2**:621, 1951.
98. Lawson, A., Rimington, C., and Searle, C. E.: Antithyroid activity of 2-carbethoxy-thio-1-methyl-glyoxaline, Lancet **2**:619, 1951.
99. Leathem, J. H.: Goitrogen-induced thyroid tumours, Ciba Foundation Colloquia on Endocrinology, vol. 12, Hormone production in endocrine tumours, Boston, 1958, Little, Brown & Company.
100. Lelong, M., Joseph, R., Canlorbe, R., Job, J. C., and Plainfosse, B.: L'hypothyroidie par anomalie congenitale de l'hormonogenese, Arch. franç. pédiat. **13**:1, 1956.
101. Libermann, D., quoted by Pitt-Rivers, R.: Physiol. Rev. **30**:194, 1950.
102. Livingston, H. J., and Livingston, S. F.: Agranulocytosis and hepatocellular jaundice; toxic reactions following propylthiouracil therapy, J.A.M.A. **135**:422, 1947.
103. McCullagh, E. P., and Cassidy, C. E.: Propylthiouracil: Four to six years follow-up of selected patients with Graves' disease, J. Clin. Endocrinol. **13**:1507, 1953.
104. McGavack, T. H.: The use of antithyroid drugs in hyperthyroidism, M. Clin. North America **36**:603, 1952.
105. McGavack, T. H.: Methimazole and carbimazole in hyperthyroidism; comparison by double blind technique, Am. J. M. Sc. **238**: 1, 1959.
106. McGavack, T. H., Chevalley, J., Reckendorf, H. K., and Haar, H. O.: Clinical experiences with carbimazole (1-methyl-2-carbethoxy-thioimidazole) in the treatment of hyperthyroidism, J. Clin. Endocrinol. **16**:887, 1956.
107. McGinty, D. A., Sharp, E. A., Dill, W. A., and Rawson, R. W.: Excretion studies on thiouracil and its 6-benzyl and 6-*n*-propyl and 6-methyl derivatives in man, J. Clin. Endocrinol. **8**:1043, 1948.
108. McGirr, E. M.: Sporadic goitrous cretinism, Brit. M. Bull. **16**:113, 1960.
109. McGirr, E. M., Hutchison, J. H., and Clement, W. E.: Sporadic goitrous cretinism. Dehalogenase deficiency in the thyroid gland of goitrous cretins and in heterozygous carriers, Lancet **2**:823, 1959.
110. MacGregor, A., and Goodwin, J. F.: Antithyroid drugs in pregnancy, Lancet **2**:89, 1953.
111. MacGregor, A. G., and Miller, H.: Antithyroid activity of 2-carbethoxy-thio-1-methyl-glyoxaline, Lancet **1**:881, 1953.
112. MacKenzie, C. G.: Endemic goiter. IV. Experimental goiter, Fed. Proc. **17**:suppl. 2: 72, 1958.
113. MacKenzie, C. G., and MacKenzie, J. B.: Effect of sulfonamides and thioureas on the

thyroid gland and basal metabolism, Endocrinology **32**:185-209, 1943.

114. MacKenzie, J. B., and MacKenzie, C. G.: The effect of "sulfa" drugs on the thyroid gland in rats and mice, Fed. Proc. **1**:122-123, 1942.
115. MacKenzie, J. B., MacKenzie, C. G., and McCollum, E. V.: The effect of sulfanilylguanidine on the thyroid of the rat, Science **94**:518-519, 1941.
116. Marine, D., and Lenhart, C. H.: Effects of the administration or the withholding of iodine-containing compounds in normal, colloid or actively hyperplastic (parenchymatous) thyroids of dogs, Arch. Int. Med. **8**:265, 1911.
117. Marine, D., Baumann, E. J., Spence, A. W., and Cipra, A.: Further studies on etiology of goiter with particular reference to action of cyanides, Proc. Soc. Exper. Biol. & Med. **29**: 772-775, 1932.
118. Meulengracht, E., and Kjerulf-Jensen, K.: Prolonged methylthiouracil treatment in thyrotoxicosis. Results after cessation of the treatment, J. Clin. Endocrinol. **8**:1060, 1948.
119. Michel, R.: Recent progress in the physiology and biochemistry of thyroid hormones, Am. J. Med. **20**:670, 1956.
120. Mirrer, G. P.: Hypoprothrombinemia following use of propylthiouracil, Ann. Int. Med. **43**:1124, 1955.
121. Moeschlin, S.: Agranulocytosis due to sensitivity to drugs, *in* Rosenheim, M. L., and Moulton, R., editors: Sensitivity reactions to drugs. A symposium, Oxford, 1958, Blackwell Scientific Publications.
122. Morton, M. E., Perlman, I., Anderson, E., and Chaikoff, I. L.: Radioactive iodine as an indicator of the metabolism of iodine. V. The effects of hypophysectomy on the distribution of labeled thyroxine and diiodotyrosine in thyroid gland and plasma, Endocrinology **30**:495, 1942.
123. Mosier, H. D., Blizzard, R. M., and Wilkins, L.: Congenital defects in the biosynthesis of thyroid hormone, Pediatrics **21**:248, 1958.
124. Paley, K. R., Sobel, E. S., and Yalow, R. S.: Effect of oral and intravenous cobalt chloride on thyroid function, J. Clin. Endocrinol. **18**: 850, 1958.
125. Paris, J., McConahey, W. M., Owen, C. A., Jr., Woolner, L. B., and Balin, R. C.: Iodine goiter, J. Clin. Endocrinol. **20**:57, 1960.
126. Peterson, R. R., and Young, W. C.: The problem of placental permeability for thyrotropin, propylthiouracil, and thyroxine in the guinea pig, Endocrinology **50**:218, 1952.
127. Pitt-Rivers, R.: The oxidation of diiodotyrosine derivatives, Biochem. J. **43**:223, 1948.
128. Pitt-Rivers, R.: Mode of action of antithyroid compounds, Physiol. Rev. **30**:194, 1950.
129. Pitt-Rivers, R.: Some factors that affect thyroid hormone synthesis, Ann. New York Acad. Med. **86**:362, 1960.
130. Pitt-Rivers, R., and Tata, J. R.: The thyroid hormones, London, 1959, Pergamon Press Ltd.
131. Plummer, H. G.: Results of administering iodine to patients having exophthalmic goiter, J.A.M.A. **80**:1955, 1923.
132. Poate, H. R. G.: Final results of long-term therapy with methyl-thiouracil in thyrotoxicosis, M. J. Australia **1**:139, 1950.
133. Poate, H.: Thyrotoxicosis treated with 2-carbethoxythio-1-methyl-glyoxaline (NeoMercazole), Lancet **1**:879, 1953.
134. Popper, H., and Schaffner, F.: Drug induced hepatic injury, Ann. Int. Med. **51**:1230, 1959.
135. Proceedings of a conference on endemic goiter, Fed. Proc. **17**: suppl. 2:57, 1958.
136. Querido, A., Stanbury, J. B., Kassenaar, A. A. H., and Meijer, J. W. A.: The metabolism of iodotyrosine. III. Di-iodotyrosine deshalogenating activity of human thyroid tissue, J. Clin. Endocrinol. **16**:1096, 1956.
137. Rawson, R. W., Rall, J. E., and Sonenberg, M.: The hormones, *in* Pincus, G., and Thimann, K. V., editors: Recent progress in hormone research, New York, 1955, Academic Press Inc., vol. III, p. 433.
138. Rawson, R. W., Moore, F. D., Peacock, W., Means, J. H., Cope, O., and Riddell, C. B.: Effect of iodine on the thyroid gland in Graves' disease when given in conjunction with thiouracil—A two-action theory of iodine, J. Clin. Invest. **24**:869, 1945.
139. Renold, A. E., Winegrad, A. I., Froesch, E. R., and Thorn, G. W.: Studies on the site of action of the arylsulfonylureas in man, Metabolism **5**:757, 1956.
140. Richards, J. B., and Ingbar, S. H.: The effects of propylthiouracil and perchlorate on the biogenesis of thyroid hormone, Endocrinology **65**:198, 1959.
141. Richardson, J. S., Sarkany, I., and Campbell, C. D.: Fatal case of marrow aplasia after treatment with carbimazole, Brit. M. J. **1**:364, 1954.
142. Richter, C. P., and Clisby, K. H.: Toxic effects of the bitter-tasting phenilthiocarbamide, Arch. Path. **33**:46-57, 1942.
143. Riker, W. F., and Wescoe, W. C.: The pharmacology and therapeutic application of antithyroid compounds, Am. J. M. Sc. **210**: 663, 1945.
144. Roche, M., and Layrisse, M.: Effect of cobalt on thyroidal uptake of $I^{131}$, J. Clin. Endocrinol. **16**:831, 1956.
145. Roche, J., Michel, R., Wolf, W., and Nunez, J.: Sur deux nouveaux constituents hormonaux du corps thyroide, la 3:3′-diiodothyronine et la 3:3′:5′-triiodothyronine, Biochim. et biophys. acta **19**:308, 1956.

146. Roche, M., Venanzi, F., Vera, J., Coll, E., Spinetti-Berti, M., Mendez-Martinez, J., Gerardi, A., and Forero, J.: Endemic goiter in Venezuela studied with $I^{131}$, J. Clin. Endocrinol. **17**:99, 1957.
147. Rosenberg, I. N.: The antithyroid activity of some compounds that inhibit peroxidase, Science **116**:503-505, 1952.
148. Sarcione, E. J., and Harrett, H. W.: Antithyroid activity of 5-iodo-2-thiouracil, Endocrinology **63**:142, 1958.
149. Schneider, W. C., and Halverstadt, I. F.: The dipole moments of thiouracil and some derivatives, J. Am. Chem. Soc. **70**:2626-2631, 1948.
150. Selenkow, H. A., and Marcus, F. I.: Masked hyperthyroidism and heart disease, M. Clin. North America **44**:1305, 1960.
151. Selenkow, H. A., Rivera, A., and Thorn, G. W.: The effects of amphenone on thyroid function in man, J. Clin. Endocrinol. **17**:1131, 1957.
152. Sensitivity reactions to drugs, Rosenheim, M. L., and Moulton, R., editors: A symposium, Oxford, 1958, Blackwell Scientific Publications.
153. Shipp, J. C.: Jaundice during methimazole (Tapazole) administration, Ann. Int. Med. **42**:701, 1955.
154. Slingerland, D. W.: The influence of various factors on the uptake of iodine by the thyroid gland, J. Clin. Endocrinol. **15**:131, 1955.
155. Slingerland, D. W., Graham, D. E., Josephs, R. K., Mulvey, P. F., Jr., Trakas, A. P., and Yamazaki, E.: The effect of propylthiouracil on the conversion of monoiodotyrosine to diiodotyrosine, Endocrinology **65**:178, 1959.
156. Solomon, D. H.: Factors affecting the fractional rate of release of radioiodine from the thyroid gland in man, Metabolism **5**:667, 1956.
157. Solomon, S., and Rubenfeld, S.: Chronic lymphatic leukemia and giant follicular lymphoblastoma after $I^{131}$ therapy, Arch. Int. Med. **106**:428, 1960.
158. Solomon, D. H., Beck, J. C., Vanderlaan, W. P., and Astwood, E. B.: Prognosis of hyperthyroidism treated with antithyroid drugs, J.A.M.A. **152**:201, 1953.
159. Specht, N., and Boehme, E.: Death due to agranulocytosis induced by methimazole therapy, J.A.M.A. **149**:1010, 1952.
160. Spector, E., and Shideman, F. E.: Metabolism of thiopyrimidine derivatives: Thiomylal, thiopental and thiouracil, Biochem. Pharmacol. **2**:182-196, 1958-1959.
161. Stanbury, J. B.: Cretinism with goiter, J. Clin. Endocrinol. **11**:740, 1951.
162. Stanbury, J. B., and Hedge, A. N.: A study of a family of goitrous cretins, J. Clin. Endocrinol. **10**:1471, 1950.
163. Stanbury, J. B., Meijer, J. W. A., and Kassenaar, A. A. H.: The metabolism of iodotyrosines. II. The metabolism of mono- and diiodotyrosine in certain patients with familial goiter, J. Clin. Endocrinol. **16**:848, 1956.
164. Stanbury, J. B., Ohela, K., and Pitt-Rivers, R.: The metabolism of iodine in two goitrous cretins compared with that in two patients receiving methimazole, J. Clin. Endocrinol. **15**:54, 1955.
165. Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S.: The metabolic basis of inherited diseases, New York, 1960, McGraw-Hill Book Co., Inc.
166. Stanbury, J. B., Kassenaar, A. A. H., Meijer, J. W. A., and Terpstra, J.: The metabolism of iodotyrosines. I. The fate of mono- and diiodotyrosine in normal subjects and in patients with various diseases, J. Clin. Endocrinol. **16**:735, 1956.
167. Stanley, M. M., and Astwood, E. B.: Determination of relative activities of antithyroid compounds in man using radioactive iodine, Endocrinology **41**:66, 1947.
168. Starr, P.: Modern medical management of thyroid diseases, M. Clin. North America **43**: 1071, 1959.
169. Starr, P., Petit, D. W., Meister, L., and Stirrett, R. L.: Therapeutic studies in hyperthyroidism: Propylthiouracil, J. Clin. Endocrinol. **9**:330, 1949.
170. Tong, W., Taurog, A., and Chaikoff, I. L.: Activation of the iodinating system in sheep thyroid particulate fractions by flavin cofactors, J. Biol. Chem. **227**:773, 1957.
171. Taurog, A., Potter, G. D., and Chaikoff, I. L.: Conversion of inorganic $I^{131}$ to organic $I^{131}$ by cell free preparations of thyroid tissue, J. Biol. Chem. **213**:119, 1955.
172. Tranquada, R. E., Solomon, J. B., and Greene, R.: The effect of oral hypoglycemic agents on thyroid function in the rat, Endocrinology **67**:293, 1960.
173. Trotter, W. R.: Some recent developments in the pharmacology of the antithyroid compounds, J. Pharm. & Pharmacol. **1**:65, 1949.
174. Vanderlaan, W. P., and Bissel, A.: Effects of propylthiouracil and of potassium thiocyanate on the uptake of iodide by the thyroid gland of the rat, Endocrinology **391**: 157, 1946.
175. Vanderlaan, W. P., and Caplan, R.: On the mode of action of iodothiouracil, J. Clin. Endocrinol. **14**:232, 1954.
176. Vanderlaan, W. P., and Storrie, V. M.: A survey of the factors controlling thyroid function with special reference to newer views on antithyroid substances, Pharmacol. Rev. **7**:301-334, 1955.
177. Vanderlaan, W. P., and Vanderlaan, J. E.:

Some factors in the metabolism of iodine by the thyroid gland, West. J. Surg. **55**:10, 1947.

178. van Wyk, J. J., Grumbach, M. M., Shepard, T. H., and Wilkins, L.: The treatment of hyperthyroidism in childhood with thiouracil drugs, Pediatrics **17**:221, 1956.
179. Vetter, H., and Hofer, R.: Acute leukemia following radioiodine therapy of thyrotoxicosis, Brit. J. Radiol. **32**:263, 1959.
180. von Mutzenbecher, P.: Über die Bildung von Thyroxin aus Dijodtyrosin, Ztschr. phys. Chem. **261**:253, 1939.
181. Wayne, E. J.: Clinical and metabolic studies in thyroid disease, Brit. M. J. **1**:1-11, 78-79, 1960.
182. Webster, B., and Chesney, A. M.: Endemic goiter in rabbits. III. Effect of administration of iodine, Bull. Johns Hopkins Hosp. **43**:291, 1928.
183. Werner, S. C.: Response to triiodothyronine as index of persistence of disease in thyroid remnant of patients in remission from hyperthyroidism, J. Clin. Invest. **35**:57, 1956.
184. Werner, S. C., Block, R. J., Mandl, R. H., and Kassenaar, A. A. H.: Pathogenesis of a case of congenital goiter with abnormally high levels of SPI and with mono- and diiodotyrosine, J. Clin. Endocrinol. **17**:817, 1957.
185. Wilkins, L.: The diagnosis and treatment of endocrine disorders in childhood and adolescence, Springfield, Ill., 1957, Charles C Thomas, Publisher, p. 135.
186. Williams, R. H.: Further studies of the absorption, distribution and elimination of thiouracil, J. Clin. Endocrinol. **4**:385, 1944.
187. Williams, R. H.: Selection of therapy for individual patients with thyrotoxicosis, J.A. M.A. **139**:1064, 1949.
188. Williams, R. H., and Kay, G. A.: Thiouracils and thioureas; comparisons of the absorption, distribution, destruction and excretion, Arch. Int. Med. **80**:37, 1947.
189. Williams, R. H., Kay, G. A., and Jandolff, B. J.: Thiouracil; its absorption, distribution and excretion, J. Clin. Invest. **23**:613, 1944.
190. Wilson, E. H., and Asper, S. P.: The role of x-ray therapy to the neck region in the production of thyroid cancer in young people, A.M.A. Arch. Int. Med. **105**:244, 1960.
191. Wing, E. G., and Asper, S. P.: Observations on the use of propylthiouracil in hyperthyroidism with special reference to long-term treatment, Bull. Johns Hopkins Hosp. **90**:201, 1952.
192. Wolff, J., Chaikoff, I. L., Taurog, A., and Rubin, L.: The disturbance of iodine metabolism produced by thiocyanate; the mechanism of its goitrogenic action with radioactive iodine as indicator, Endocrinology **39**:140, 1946.
193. Wollman, S.: Concentration and organic binding of radioiodine by the thyroid gland, Ann. New York Acad. Med. **86**:354, 1960.
194. Wood, J. L., and Kingsland, N.: Labeled sulfur uptake by thyroids of rats with low plasma thiocyanate levels, J. Biol. Chem. **185**: 833-837, 1950.
195. Wood, J. L., and Williams, E. F.: The metabolism of thiocyanate in the rat and its inhibition by propylthiouracil, J. Biol. Chem. **177**:59, 1949.
196. Wyngaarden, J. B., Stanbury, J. B., and Rapp, B.: The effects of iodide, perchlorate, thiocyanate and nitrate administration upon the iodide concentration mechanism of the rat thyroid, Endocrinology **52**:568, 1953.
197. Wyngaarden, J. B., Wright, B. M., and Ways, R.: The effect of certain anions upon the accumulation and retention of iodide by the thyroid gland, Endocrinology **50**:537, 1952.

# Therapeutic trends in the treatment of barbiturate poisoning

## The Scandinavian method

*The "Scandinavian method" of treating barbiturate poisoning is presented. As a result of close and constant attention to the support of vital function (the cardiovascular system, respiration, renal function, electrolyte homeostasis) and the prevention of infection, the mortality rate from barbiturate poisoning in our clinics has been brought down to 1.5 per cent whereas previously it was over 10 per cent. The details of this method, the history of its development, and the basis of its use are explained.*

**Carl Clemmesen and Eric Nilsson** *Copenhagen, Denmark, and Lund, Sweden*
*Psychiatric Department, Bispebjergs Hospital, Copenhagen, and University Hospital, Lund*

The present review of the treatment of barbiturate poisoning does not embrace the world-wide literature on this subject. A review of this sort should perhaps have such a background, but the countless articles and publications unfortunately very often have widely different approaches and employ varying standards of definition regarding coma, duration of unconsciousness, initiation of treatment, etc., which make it difficult to draw comparative conclusions from them. The writers have therefore chosen to give an account of their views with primary reference to the Scandinavian literature on this subject. This may seem a slender basis for a review, but since it refers to the largest uniformly treated series to be found in the world-wide literature, we consider it justifiable. The records of this series are to be found at the Förgiftningscentralen, Bispebjergs Hospital, Copenhagen. The layout and organization of this department will be referred to later.

Received for publication Nov. 28, 1960.

### Rising incidence

Barbiturate poisoning and its treatment have been in the limelight in the last 15 years, mainly because of the enormous increase in such cases after the Second World War. This increase has been many fold both in Sweden and in Denmark and involves not only the number of cases but often also their severity, that is to say, the doses taken in some cases have been enormous. Whether this signifies more determined suicidal attempts or a reaction to the publicly known increasing efficacy of treatment is difficult to elucidate. During the last few years, however, other drugs such as meprobamate and the phenothiazines have been used as suicidal poisons, giving rise to states which are more easily treated.

Intoxication with hypnotic drugs is a modern medical problem, interesting and

serious, and one to which both the community and the physician should be constantly alert. The mortality rates reported from various parts of the world fluctuate considerably. Thus, Isbell[7] in 1951 calculated that there were about 1,500 deaths yearly in the United States from this cause, corresponding to a mortality of 8 per cent. At the Copenhagen center, 1,500 or more cases of unconscious poisoning are treated each year, of which about 75 per cent are the result of hypnotic drugs. The collective mortality there is 1 to 2 per cent, that is, 20 to 30 patients die each year. These figures are very low, and there seems little possibility of reducing them further. A survey of the causes of death, however, gives pointers to possible therapeutic measures for the future which may enable even more of these patients to be saved, but these must depend on expansion and intensification of research. The lethal complications could perhaps be treated earlier; the connection between the circulation, renal function, and central depression could be further illuminated. Definition of the function of the adrenocortical hormones may perhaps provide the means with which to diminish the effects of the stress factor and the risk of falling blood pressure, with its consequent renal damage, and to increase the patient's ability to withstand anoxia. Earlier and better treatment of acidosis may be yet another means of reducing the mortality figures.

### Emergence of Scandinavian method

Although it is the Copenhagen center's treatment routine with which we are mainly concerned in this survey, we will nevertheless begin with a short historical review of barbiturate poisoning therapy and its development from the "stimulation" treatment of the 1930s to the methods prevailing today. Up to the middle of the 1940s, the basis of treatment was massive gastric lavage, often with suspensions of powdered carbon together with intensive use of "central analeptics." The results of this treatment showed no improvement over those during the previous 20 years, however, and the central analeptics did not fulfill the promise indicated by animal experiments. In 1942 Harstad, Möller, and Simesen[6] shed new light on the matter by showing that gastric lavage was not entirely without risk and that its therapeutic value was, to say the least, doubtful. The quantities of barbiturate which could be removed in this way were very small compared to the amount ingested, and patients were in any case usually admitted after the greater part of the barbiturate had passed on into the small intestine. It was furthermore shown that intestinal peristalsis ceased after large doses of barbiturate and that, therefore, the carbon suspension failed to reach the intestine. Not infrequently in fatal cases, carbon particles were found in the lungs at autopsy. The aspiratory pneumonia often found in such cases must therefore be designated as in part the result of treatment.

The intensive central stimulation therapy used during this era did not reduce the mortality below 20 per cent. Furthermore, this therapy gave rise to situations in which it was difficult to estimate the patient's condition. One saw a mixture of symptoms which were partly a result of the poison, partly a sequela of the comatose condition, and partly, one suspected, a result of the stimulating drug.

In 1946 Kirkegaard[8] showed that one of the most important pathophysiologic factors was peripheral circulatory collapse—shock. Most of the patients who succumbed to poisoning died in typical circulatory failure. The therapeutically important result of Kirkegaard's work was the introduction of effective measures to combat shock, and from that time onward, the mortality rate began to fall. When the further significance of a free and continuously patent airway together with the prevention of long periods of hypoxia was pointed out by Nilsson[18] in 1951, the way was opened for a more effective approach to the treatment problem. It had been shown by animal experiments that central analeptics could

cause complications in the form of convulsions, reduced cerebral oxygen tension, and postconvulsion episodes of hypoxia. By eliminating central analeptics from the treatment and rigidly observing physiologic principles in therapy, antishock measures, a free airway, and possibly the use of oxygen, the mortality rate was brought down. Through the years, this form of treatment has fulfilled its early promise. Certain adjustments have been made after the appearance of new types of complication, problems one did not see previously since the death of the patient usually forestalled their development.

This type of treatment has been referred to in the literature as "Scandinavian therapy" and also (because no central analeptics such as pentylenetetrazol and picrotoxin are used) as "pharmacotherapeutic nihilism."[10] Our regimen, which is carried out day after day until the unconscious patient is out of danger, requires intensive organization of both treatment and staff, closely comparable with the activities of the "intensive therapy" units which are now being developed in most modern hospitals. We consider that accusations of nihilism can be leveled neither at our staff, our methods, or our results, however brilliant may be the results obtained with pentylenetetrazol* or picrotoxin in animal experiments.

### Clinical evaluation

Clemmesen has described the following three stages or phases through which poisoned patients may pass after taking large doses of hypnotics.

***Induction phase.*** This is characterized by a state of physiologic imbalance brought about by the poison. Treatment must be started at once and should be directed toward the prevention of lethal complications. The sooner normal physiologic conditions can be established, the better. If too long is taken to correct the shock or to secure the requirements for normal respiration adequate to maintain $O_2$ and $CO_2$ at normal blood tensions, the patient can be lost before the real battle has started.

***The second phase.*** The patient lies in a stabilized state of coma and in approximate physiologic balance. His condition must be carefully checked at hourly intervals, and he must be under continuous observation day and night. The coma may last a considerable time. Reawakening tends to occur in waves rather than uniformly. In our experience, the longest coma to be followed by complete recovery has been 12 days.

***The third phase, reawakening.*** The patient begins to react more and more to certain stimuli. He coughs during tracheobronchial evacuation. The pharyngeal reflex returns, and he begins to make more or less coordinated movements and is sometimes very restless. He may even be quite unruly. When the pharyngeal reflex has returned satisfactorily, the patient may be allowed to begin to take fluids by mouth, but he should not be regarded as awake until he can reply to a question with an adequately articulated word. Despite his improved condition, the patient is not entirely out of danger, and he should be watched carefully during the subsequent days. The patient is still very weak and fatal complications can still develop. As a rough and ready rule, we estimate that after regaining consciousness, the patient should be closely observed for that number of 4 hour periods corresponding to the duration of his coma in days; for example, 6 days' coma requires 24 hours' postcoma observation.

### The Scandinavian routine in detail

These therapeutic measures should be judged and evaluated primarily with regard to those complications which can occur and threaten life in association with barbiturate poisoning. Admittedly, the poison itself may be the direct cause of death, but more usually it is the result of secondary complications in the circulatory, respi-

*Metrazol.

ratory, or renal system. Our treatment is therefore aimed at securing as rapid a breakdown and removal of the barbiturate as possible, together with prevention and treatment of the afore-mentioned complications.

The causes of death at the Copenhagen center can be divided roughly as follows, according to the paper published by Myschetsky[17] in 1955. Pulmonary and cardiac complications accounted for one-third each. Renal damage and irreversible shock were each responsible for one-sixth. These figures are essentially valid today. Thus the circulation, respiration and the airway, and renal function have become the foci of attention, and it is toward the maintenance of normal physiologic conditions in these that our efforts have been directed.

### Cardiovascular system

The dominant cause of death before 1945 was peripheral circulatory collapse. It is known that an overdose of barbiturate can lead to a primary fall in blood pressure, which is a feature of the poison's effect on the vasomotor center. Later comes a secondary fall in pressure—the result of the barbiturate's effect on the vascular bed of the myocardium. The patient thus develops a typical shock syndrome, with low blood pressure, a rapid, feeble pulse, and pale, cold, sweating skin (especially on the extremities), and usually an obvious hemoconcentration is underway. This picture is typical and is exhibited by the majority of patients on admission to hospital. Treatment is started at once. Dextran is given intravenously while blood grouping and cross matching are being done. Sometimes concentrated plasma is used instead. When frank shock is present, 2 to 3 L. of blood, dextran, and plasma may be needed to check it and to bring the patient into the more stable phase. Active treatment must not be discontinued until the patient is out of the shock phase. This question is discussed further in the section dealing with the use of arterenol and renal complications.

### Corticosteroids and the circulation

In many other fields in medicine, when the circulation begins to fail efforts are made to support the function of the suprarenal glands by the administration of corticosteroids. Of these, the water soluble preparations for intravenous use now current are of great value. It was the work of Seyle on the connection between adrenocortical function and the stresses to which a patient may be subjected that was the basis of this form of therapy. In barbiturate poisoning, the patient is severely stressed, partly by the poisoning as such and partly by the secondary shock, both factors being of special importance in this respect. It is not surprising, therefore, that the adrenocortical hormones operate particularly in the early emergency phase, tending to mitigate the stress-evoked factors. In 1952 Harslöf[5] attempted to improve the results by the use of ACTH. A dosage of 100 mg. was given daily to a number of patients; urinary hormone excretion was then followed to obtain an impression of the adrenocortical function. Occasionally, a stabilizing effect on the circulation was observed, without, however, any reduction in coma duration or any hastening of barbiturate elimination. In 1954-1955 a similar trial was made of desoxycorticosterone (DOCA).[14] Dosages of 45 to 105 mg. were given daily to similar severely intoxicated patients, and no conclusive results were obtained compared with those in a control series.

No difference was observed either in coma duration, shock duration, or the need for parenteral fluids. However, urinalysis demonstrated that there occurred a natural response of the suprarenal cortex to the stress of poisoning, as with other types of stress.

With the advent of purified, water soluble hydrocortisone preparations such as those now available for intravenous use, perhaps better results may be obtained. An investigation of this question has been carried out, but as yet no results are available. However, when severe shock is present, the

intravenous preparations may be tried daily during the first few critical days, to support the otherwise standard shock therapy.

### Respiration and the airway

Pulmonary and respiratory complications have always caused large numbers of deaths with barbiturate poisoning. Previously it was usually pneumonia, but recently it has been central respiratory depression which has been the predominant factor. Prevention of pneumonia is carried out much more energetically these days, and even if occasionally there are cases of resistant staphylococcal pneumonia, even these occur less commonly than before. A rise in temperature and suspicion of pneumonia indicate prophylactic penicillin in doses of 2 to 5 million U. twice a day. The management of the unconscious patient's airway must be such that it is kept free and patent during all phases and in all situations, so that there is adequate ventilation, i.e., adequate oxygenation and removal of carbon dioxide.

The patient is turned over from one side to the other at 2 hour intervals day and night. Routine daily chest x-rays forestall atelectasis. If not dealt with, atelectasis can ,give rise to bronchopneumonia, especially in patients who are admitted to hospital long after ingestion of the barbiturate. Atelectasis is treated along anesthesiologic principles with tracheobronchial clearing and, if necessary, by bronchoscopy, and in this way it often disappears as quickly as it has arisen. Oxygen is given from time to time via a nasal catheter, or by catheter through a pharyngeal airway. If difficulty is experienced in keeping the bronchi free of secretion by ordinary means or if the secretions become excessive, an endotracheal tube is passed and left in situ. The period of intubation should not exceed 4 days, however, and if a longer period is anticipated, one should resort to tracheotomy. If the tube is allowed to remain in the trachea for longer periods, there is risk of decubitus ulceration of the tracheal wall, with subsequent adhesive tracheitis, and also a risk of laryngeal changes, with obstructive laryngitis at a later stage. The most serious respiratory problem currently is central respiratory depression, a result of the often enormous doses of drug the patient has taken and which may finally lead to respiratory failure, It is in fact not often that respiratory arrest supervenes, but when central depression is so enormous that the respiratory center, deep in the brain stem, is incapacitated, the situation is serious. Always the question has been asked: "Cannot a stimulating drug be used to reverse this central depression and thus support respiration?" Despite the assertion from many quarters that the "central analeptics" referred to previously are capable of doing this, they have never succeeded in doing so at the Copenhagen center. The only cases in which one saw any effect from convulsive analeptics such as pentylenetetrazol and picrotoxin were those in which such stimulation was not in fact needed. In the really severe cases, no benefit has been observed.

### The place of bemegride

In 1955 Shaw reported that he had discovered a real antidote to the barbiturates, and the early publications on the subject were very encouraging. Bemegride* was supposed to lighten the coma of even deeply unconscious patients and to bring them up into a "safe state," from which they could relatively quickly be roused to wakefulness. Louw and Sonne[15] in 1956 investigated bemegride treatment on a series of gravely poisoned patients, several of whom were apneic or becoming so. The first and striking experience was that bemegride brought about a tangible stimulation of respiration and a simultaneous hyperreflexia. Clemmesen[3] in 1956 considered this a noteworthy effect and one which he had never previously witnessed during his efforts to forestall or reverse apnea, a much dreaded complication. Further investiga-

*Megimide.

tions failed to confirm Shaw's findings that bemegride shortened the duration of coma, hastened the elimination of the barbiturate, and caused the patient to awaken with higher blood barbiturate levels than was possible previously. In 1956 Pedersen[21] established that the stabilizing effect of bemegride was not such as to allow of any relaxation of antishock measures and that barbiturate elimination followed the same course as in those patients who had received no bemegride. Kjaer-Larsen[9] was able to show in 1956 that a considerable number of bemegride-treated patients exhibited psychoses during convalescence. These psychoses were characterized partly by visual halucinations and partly by delirium. They were admittedly of relatively slight degree, the incidence being about 30 per cent, and spontaneous recovery usually occurred in 2 to 6 days.

After this period of research, the use of bemegride has continued in certain special cases. The early impressions remain, and the effect on respiration, even in desperately grave cases, is beyond dispute. Its properties do not lie, however, in a purely pharmacologic antagonism to barbiturates, since the effects of the latter persist. As a central analeptic, which bemegride must be regarded, it is superior to earlier preparations in that it does not cause hypertension and overtaxing of the already intoxicated myocardium, neither does it cause hyperpyrexia. The use of bemegride in patients occasionally does not, however, justify neglecting the antishock regimen, which remains the most vital factor in bringing about as normal a physiologic condition as possible during the coma period.

### Artificial ventilation—A new approach to control

Another approach to treatment if apnea or respiratory insufficiency supervenes is to institute artificial respiration. Without doubt, the best way of doing this is to use a mechanical ventilator. This type of therapy is becoming more and more common in medicine today in cases in which there is depression of the central respiratory mechanism or a peripheral affection of the respiratory musculature. The indications for beginning artificial ventilation and the estimation of its effectiveness are critical for homeostasis. In a barbiturate-intoxicated patient, satisfactory blood oxygen tension can usually be maintained by the simple use of oxygen, but whether or not carbon dioxide retention is present is much more difficult to determine. Respiratory acidosis can develop very insidiously, and the only sure means of diagnosis is serial blood gas analysis. It has up to now implied arterial puncture and much laboratory work to obtain blood $pCO_2$, pH, and standard bicarbonate determination. These requirements have perhaps hindered somewhat the progress of artificial ventilation, since the physician supervising treatment does not receive the results of these tests for some hours, by which time the clinical picture may have altered entirely. These considerations are admittedly applicable only when the decision involves a case of depressed but persisting spontaneous breathing. If total apnea presents, artificial ventilation is always indicated.

During recent years Astrup and colleagues[2] have evolved a new approach to the estimation of acid-base equivalence. From a pH determination and a nomogram which includes a base-excess (or base-deficit) curve, they were able to make important deductions. If the pH is known, $pCO_2$ and base-excess or base-deficit expressed as milliequivalents per liter of blood can be easily calculated from the curve in the nomogram. A direct answer is always obtained on the important features such as pH, $pCO_2$, and the acidotic or alkalotic tendencies in the blood. That the answer is given rapidly means that the method could be put to good use in all situations involving artificial ventilation of patients. A further advantage is that this method is a microtechnique and capillary blood only is required, thereby eliminating the need for arterial puncture. This revolu-

tionary technique will simplify the assessment of all suspected cases of acidosis to a very great extent and will facilitate the accurate control of artificial ventilation.

### Renal complications

In Myschetsky's[17] survey in 1955, one-sixth of the deaths from barbiturate poisoning resulted from renal complications. In the middle 1950s, when these complications reached their peak at the Copenhagen center, they proved among the most difficult to handle. Anuria and uremia were encountered not only in comatose patients but also in those who were at a later stage and had regained consciousness.

Kidney failure is a most serious complication, since the elimination of barbiturate is dependent on its excretion by this organ. In an elegant demonstration, Lous[13] has shown how the blood concentrations of different barbiturates behave during anuria. Barbital and phenobarbital, which are eliminated by the kidneys completely and partially, respectively, show no alteration in blood levels, while preparations such as apobarbital, amytal, and hexobarbital show falls in concentration despite anuria.

In order to elucidate the risk of anuria and the patient's prognosis, in 1956 Paerregaard[20] followed the creatinine clearance in a number of cases of severe poisoning; 51 patients were involved, and creatinine clearance was done daily for the first 4 days after admission. The lowest 24 hour figure observed during these 4 days was made the basis for prognostic purposes. It could then be predicted that if in a given patient this value remained over 40 ml., survival was likely. Those with a value of 10 ml. or below developed uremia, and of these, 50 per cent died. The value of this examination in severe cases was thus established, and it has continued in routine use. Those in charge of the patient are thereby enabled to estimate the likelihood of renal complications and to make prognosis for survival.

**Table I.** *Case incidence and mortality, 1948-1959, Copenhagen center*

| *Year* | *No. of cases* | *No. of deaths* | *Mortality rate (%)* |
|---|---|---|---|
| 1948 | 802 | 96 | 12.0 |
| 1949 | 1,041 | 63 | 6.1 |
| 1950 | 1,288 | 48 | 3.7 |
| 1951 | 1,276 | 21 | 1.6 |
| 1952 | 1,115 | 25 | 2.2 |
| 1953 | 1,790 | 37 | 2.1 |
| 1954 | 1,807 | 41 | 2.3 |
| 1955 | 1,913 | 32 | 1.7 |
| 1956 | 1,837 | 20 | 1.1 |
| 1957 | 1,942 | 25 | 1.3 |
| 1958 | 1,827 | 28 | 1.5 |
| 1959 | 1,959 | 29 | 1.5 |

In attempting to avoid such complications, which no doubt are the sequelae of irreversible shock and hypotonia together with prolonged hypoxia, management has proceeded along the following lines. If the shock has not abated after 1,000 ml. dextran, plasma, or blood, arterenol is added to the infusion to maintain blood pressure and renal perfusion pressure. It is recognized that arterenol causes renal vasoconstriction in normal persons, but according to von Euler,[4] when circulatory depression with hypotension and failing renal filtration is present, the latter can be improved by arterenol. This indicates that in such situations, it is the blood pressure which is the vital factor, and by raising it, renal function is improved despite a certain degree of vasoconstriction. The clinical experience with poisoned patients confirms this, and since introduction of this method, the incidence of anuria and uremia has declined.

Despite this treatment, some patients still become anuric. These require dialysis. For success with dialysis, it is highly desirable to have access to and cooperation with a specialized "artificial kidney" unit. Eighteen anuric patients were thus treated in 1955, and of these desperately ill persons, 8 have been saved. The significance of this approach has been discussed by Alwall, Lindgren, and Lunderqvist[1] and by Kyle.[11]

Dialysis has also been discussed as a potentially routine method of securing rapid elimination of barbiturates from the

body. However, in view of the good results we have obtained with our present methods, this innovation does not seem justified. Conversely, the idea of using not an artificial kidney but the patient's own as a dialyzing mechanism has long been discussed. That is, by stimulating renal activity, an enormous diuresis is provoked, thus securing hastened elimination of barbiturate, shortening of coma, and reduced risk of complications. As long ago as 1949 Ohlsson[19] introduced the idea of blood lavage, that is, massive infusions plus a diuretic. He was able to demonstrate thereby a marked increase in the rate of barbiturate elimination.

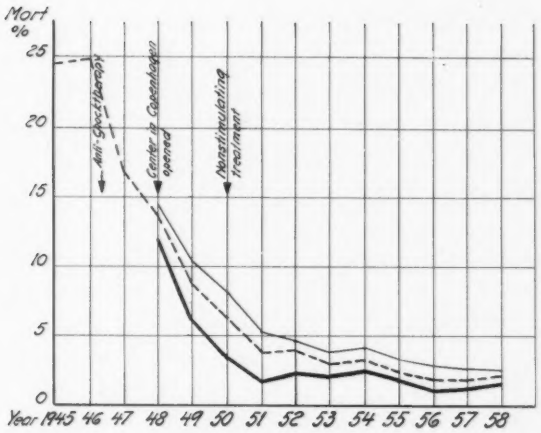



**Fig. 1.** ------, Mortality in Denmark.
———, Mortality at Copenhagen center.
———, Mortality in Denmark excluding Copenhagen center.

### Alkalinization and forced diuresis

Waddel and Butler[22] showed that increased diuresis could be obtained by pushing the pH of the blood toward alkalinity. Mollaret and associates[16] were also able to show a significant increase in phenobarbital excretion by the same means. Furthermore, it was shown that alkalinization of the blood brought about a rise in blood barbiturate levels, signifying a mobilization and transfer of the drug from cell to plasma. This was the rationale for Mollaret's regimen in the treatment of barbiturate poisoning, and it was applied especially to those cases in which phenobarbital was the responsible agent. Alkalinization was brought about by the daily administration of from 3 to 4 L. of bicarbonate solution. The relatively large amounts of fluid used, together with increased pH, thus brought about a free diuresis and aided elimination of the poison. Their use of this technique gave very satisfactory results.

At the Copenhagen center, this method was employed during 1960 by Lassen,[12] with the addition of a diuretic to increase the diuresis still further. He treated 14 severe cases in this way, pushing the diuresis as high as 10 or 11 L. per day. By comparing these cases with others not so treated yet equally severe, or by occasionally using cases as their own control where there had been repeated suicidal attempts, he was able to conclude that this regimen reduced the length of coma by approximately 50 per cent. It is doubtful, however, whether this method should be adopted routinely in all cases of barbiturate poisoning. As such though, it shows every promise, particularly where barbital and phenobarbital are involved, provided the technical details can be simplified without reducing its effectiveness. Lassen also points out that the majority of agents with a central depressive effect are easily able to pass the blood-brain barrier, and therefore it is justifiable to anticipate increased elimination of these too by the blood lavage technique.

### Our methods summarized

The following is a rough summary of the routine treatment procedure as carried out at the Copenhagen center today.

1. The patient is unconscious on admission. Large scale gastric washouts are not resorted to. If there is evidence that the drug was taken an hour previously or less, simple aspiration of the gastric contents may be carried out.

2. The airway is checked and cleaned out, and if the patient's reflexes permit, some form of pharyngeal airway or endotracheal tube is passed.

3. Hematologic investigations are done, i.e., hemoglobin, hematocrit, blood barbiturate concentration, and paper chromatography, to determine the responsible agent. Urgent blood grouping and cross matching are also carried out.

4. The condition of the circulatory and respiratory systems is estimated, and their continuous recording is begun. All data are recorded on a special chart so that the medical attendant has a continuous record of the progress of the patient. Blood pressure, pulse, respiration, and temperature are the chief notations. Hemoglobin concentration is estimated at 4 hour intervals to provide early warning of hemoconcentration, and urinary output is followed for the same reason.

5. Chest x-rays are performed at least once a day so that atelectasis and incipient pneumonia may not pass unheeded. The patient is turned every 2 hours and the airway is cleaned out by tracheobronchial aspiration or bronchoscopy, as indicated. If respiration ceases or becomes depressed, bemegride may be tried or artificial ventilation put in use.

6. Creatinine clearance is determined once a day during the first 4 days if any doubt exists, and this, together with urinary output measurements, forewarns of the onset of renal complications, oliguria, anuria, uremia, etc.

As previously mentioned, this routine has been referred to as the "Scandinavian method." It requires above all an organization and a staff capable of carrying out treatment along the lines indicated above and demands intensive, around-the-clock effort. Such an organization can best be compared with the intensive therapy units which are today being developed in larger hospitals the world over. Such units are costly, but the expense is well repayed in the great saving of human life thereby engendered.

### Results and conclusions

Table I and Fig. 1 set out the results achieved by the methods of the Copenhagen center. Since the introduction of antishock measures in 1946 and the exclusion of stimulation methods in 1950, at which point our more physiologic regimen was introduced, mortality figures have been as low as 1 to 2 per cent. The figure of 20 per cent prevailed previously despite utilization of a program of intensive central stimulation.

Until such time as better results than those with our methods are forthcoming, we decline to accept the charge of nihilism. We are in fact inclined to feel optimistic rather than nihilistic, not so optimistic, however, that we consider the mortality figures can ever be reduced to zero, since there will always be those patients who, by taking enormous doses of drug, ensure their suicide, and there will always be those who have, by the time of their admission to hospital, developed complications that are beyond the reach of even the most energetic treatment. These patients will continue to pose therapeutic problems.

### *References*

1. Alwall, N., Lindgren, P., and Lunderqvist, A.: On the artificial kidney, Acta med. scandinav. **143**:299, 1952.
2. Astrup, P., Siggaard-Andersen, O., Jörgensen, K., and Engel, K.: The acid-base metabolism. A new approach, Lancet **1**:1035, 1960.
3. Clemmesen, C.: Effect of Megimide and amiphenazol on respiratory paresis, Lancet **2**:966, 1956.
4. von Euler, U. S.: Epinephrine and norepinephrine, actions and use in man, Clin. Pharmacol. & Therap. **1**:65, 1960.
5. Harslöf, E.: Barbitursyreforgiftninger og ACTH, Ugesk. laeger **114**:601, 1952.
6. Harstad, E., Möller, K., and Simesen, M.: Über den Wert der Magenspülung bei der Behandlung von akuten Vergiftungen, Acta med. scandinav. **112**:478, 1942.
7. Isbell, H.: Acute and chronic barbiturate intoxication, Veterans Admin. Tech. Bull. 10-76, pp. 1-13, 1956.
8. Kirkegaard, Å.: Den svaere akutte barbitursyre-forgiftning, Thesis, 1951, University of Copenhagen.
9. Kjaer-Larsen, J.: Delirious psychosis and convulsions due to Megimide, Lancet **2**:967, 1956.
10. Koppanyi, T., and Fazekas, J. F.: Pharmaco-

therapeutical nihilism in the treatment of barbiturate poisoning. Progress of medical science, Am. J. M. Sc. **224**:577, 1952.

11. Kyle, L. H.: The application of hemodialysis to the treatment of barbiturate poisoning, J. Clin. Invest. **32**:364, 1953.
12. Lassen, N. A.: Treatment of severe acute barbiturate poisoning by forced diuresis and alkalinisation of the urine, Lancet **2**:338, 1960.
13. Lous, P.: Barbituric acid concentration in serum from patients with severe acute poisoning, Acta pharmacol. et toxicol. **10**:261, 1954.
14. Louw, A.: Treatment of acute barbituric acid poisoning, Dan. M. Bull. **5**:137, 1958.
15. Louw, A., and Sonne, L. M.: Megimide in the treatment of barbituric acid poisoning, Lancet **2**:961, 1956.
16. Mollaret, P., Rapin, M., Pocidalo, J., and Monsallier, J. F.: Le traitement de l'intoxication barbiturique aiguë, Presse méd. **67**:1435, 1959.
17. Myschetzky, A.: Dødsårsager ved akutte Barbitursyreforgiftninger, Ugesk. laeger **117**:195, 1955.
18. Nilsson, S. E. H.: Treatment of acute barbiturate poisoning. A modified clinical approach. Thesis, Acta med. scandinav., suppl. 253, 1951.
19. Ohlsson, W. T. L.: Blodsköljningsbehandling vid svår akut barbituratförgiftning, Nord. med. **42**:1471, 1949.
20. Paerregaard, G.: Nyrefunktionen ved akut Barbitursyreforgiftning, Ugesk. laeger **118**:778, 1956.
21. Pedersen, J.: Arousing effect of Megimide and amiphenazol in allylpropymal-poisoning, Lancet **2**:965, 1956.
22. Waddell, W. J., and Butler, T. C.: J. Clin. Invest. **36**:1217, 1957.

*The effects of a variety of hypotensive agents (hydralazine [Apresoline], hexamethonium, Rauwolfia, Veratrum alkaloids, and the low-sodium diet), studied in a series of hospitalized and ambulatory patients with hypertensive vascular disease of all degrees of severity, have clearly indicated that the usual method of evaluation fails to present an accurate picture of the specific role of these drugs in treatment regimens. When the influence of variables created by nonpharmacological forces impinging upon the patient and the physician during the experimental or therapeutic situation was systematically examined, it was found that these factors were capable of affecting blood pressure and symptoms in a fashion that potentiated, mimicked, and occasionally masked specific pharmacological activity.*

From "Consideration of Multiple Variables in Evaluation of Hypotensive Drugs" by Alvin P. Shapiro, Journal of the American Medical Association, vol. 160, p. 28, 1956.

# Clinical pharmacology in industrial medicine

*The industrial medical department provides a satisfactory setting for research in clinical pharmacology. It offers an opportunity not only for evaluating the therapeutic efficacy of many drugs but also for the study of their effects on "normal" subjects. Among its advantages is the availability of suitable human subjects who can participate in these studies without loss of income, who are readily accessible for any necessary follow-up, and whose work performance may provide a satisfactory index of the effects of certain drugs. The scientific requirements and standards for clinical pharmacologic research in industry are no different from those governing similar research in other settings. Certain problems, however, arise out of the necessity of conducting the research within the framework of the primary objective of the occupational health program: to maintain optimal productivity by the promotion of better employee health.*

**Leon J. Warshaw, M.D., F.A.C.P.*** *New York, N. Y.*

Biologic research has long been a fundamental part of industrial medicine. The profusion of new chemicals and materials that present potential health hazards has necessitated continuing research into their toxic propensities and methods of obviating their noxious effects. An increasing awareness of the threat of toxic occupational exposures has stimulated research into techniques of monitoring and controlling industrial processes. In addition, physicians, chemists, physicists, toxicologists, industrial hygienists, and other trained scientists have been actively seeking the knowledge required for the essential progress in the diagnosis, treatment, and prevention of occupational diseases. In recent years, industrial medicine has also received increasing attention as a setting for research in clinical pharmacology. The necessity for conducting controlled studies in accord with the primary objective of occupational medicine, the maintenance of optimal productivity through the promotion of better health, and within the framework of "good" personnel policies and labor-management relations creates special problems beyond those encountered in the usual forms of human experimentation. In this article, some of these problems and their resolution will be discussed in the light of the experiences of the author.

The scientific standards for investigations in clinical pharmacology are the same regardless of where they are conducted.

Supported in part by grants from the National Institute of Mental Health, U. S. Public Health Service, Bethesda, Md. (M-2225 and MY 2922-C1), and from the Upjohn Company, Kalamazoo, Mich.

Received for publication July 17, 1960.

*Consultant in Occupational Health; Medical Director, United Artists Corporation; Medical Director, Paramount Pictures Corporation.

The physicians, nurses, and technicians involved in a study must be trained in performing the required procedures, making the necessary observations, and recording the data on a research level. An experienced clinical pharmacologist and an accomplished biostatistician are needed to supervise the design of the experiment and the evaluation of the data. Such individuals are not usually found in the average industrial medical department. These services can sometimes be procured through collaboration with a university pharmacology department or with one of the clinical research departments maintained by most major pharmaceutical companies.

To conduct drug evaluations in an industrial medical department, certain conditions must be met that are peculiar to this setting. A primary prerequisite is permission to use the medical facility for research. No investigative activity of any kind should be undertaken without the knowledge and explicit consent of management. The industrial physician must make sure that the purpose of the study and the general nature of the procedures involved are completely understood and acceptable to the company executive to whom he reports and also to the director of personnel before any experiments are initiated.

The industrial physician will find extreme difficulty in recruiting suitable subjects unless there is a well-established medical program that has earned the confidence and respect of the employees it serves. Unlike hospital practice in which a physician is automatically endowed with the prestige of the institution, the industrial physician must earn his status by his treatment of the employees and their problems. Even after a project is underway, he must never allow his research activities to appear to take precedence over their medical problems. The company "grapevine," a communication miracle still unmatched by electronic scientists, will promptly convert the suspicion and displeasure of a few employees into a general lack of cooperation with his research activities.

Since the medical department is part of management, it cannot serve as a setting for research unless the company's over-all personnel relations are satisfactory. The physician must be particularly careful to postpone his studies at times of strife between management and organized labor, for it might be most embarrassing for him if his research activities became a controversial item on the collective bargaining table. Also, since participation in these studies should always be voluntary, the individual employee must feel free to terminate his participation at any time without fear of reprisal or subsequent rejection by the medical department.

The importance of adequate follow-up procedures as a part of drug evaluations is well established. Many an apparent therapeutic triumph has proved to have been only a placebo effect when the follow-up demonstrated a return of signs or symptoms that would not have occurred if the experimental therapy had been truly efficacious. The industrial physician is in an advantageous position in this respect since his subjects are usually readily available to him. However, a follow-up planned as an integral part of the experiment will be successful only if the medical department has a well-established policy of routinely following up many of the patients it treats. If such follow-up is normally a rare event, the necessity of pursuing it as part of a drug investigation may bring undue prominence to the study and give the impression that the medical department is not interested in the employees except as subjects for research. Such an inference cannot only threaten its future research activities but may undermine the employees' confidence, upon which the successful operation of the medical department inevitably depends.

Since the diagnosis and treatment of most of the employees' ailments are primarily the responsibility of their private physicians, care must be taken to avoid any infringement on this relationship by the clinical studies. If interruption of an established maintenance regimen is required to

observe a clear-cut response to a particular drug, the prior consent of the subject's personal physician must be obtained. This is usually readily obtainable and, on occasion, the private practitioner may even agree to report certain observations that may be helpful adjuncts to the study. He must be allowed, however, to retain the prerogative of prescribing whatever he deems necessary for his patient's welfare regardless of its effect on the latter's participation in the research study.

New drugs should not be administered to volunteers in industry unless intensive studies under carefully controlled conditions, in human subjects as well as in appropriate laboratory animals, have demonstrated them to be reasonably free from dangerous effects. Furthermore, extreme caution must be exercised to minimize the possibility of drug effects that will threaten the employee subject's safety, or that of his co-workers and the general public, or are likely to unduly diminish his productivity. This applies particularly to drugs that can impair vision, cause drowsiness, or lengthen reaction time. The doses to be administered should be small enough, as determined by prior pilot studies, that such reactions will be the exception rather than the rule. The subject should also be warned of the possibility that they may occur and be advised how to counteract them. Since the necessity of a routine warning that reactions *may* occur inevitably assures that at least a few of the more suggestible individuals will experience them, certain employees should never be allowed to serve as subjects in studies of drugs with these potentialities: operators of motor vehicles, mobile equipment (i.e., overhead cranes, power shovels, fork trucks), and power machines (i.e., saws, lathes, presses) and those whose job safety demands physical agility and muscular coordination (i.e., riggers, scaffold and certain construction workers). This may introduce an element of bias into the study that should be considered in evaluating the data.

Pharmacologic studies in industrial medicine, as elsewhere, frequently derive their validity from the employment of the double blind technique by which, as a rule, the actions of the drug under investigation are compared to those of a placebo. In many instances, however, an active drug of established effectiveness may be used instead of the placebo for the control observations. This will obviate the possibility of a complaint about knowingly giving an inert drug to employees in need of effective medication. It should be emphasized in passing that the special considerations already mentioned make it particularly important that the code by which the various medications are labeled be in the hands of an easily accessible individual, preferably a member of the medical department where the research is in progress.

Although much has been written about the use of placebos, there are few positive statements about the advisability of informing the subject that he may be given one. Some investigators feel that this fact should always be concealed from him. Personal experience, however, has amply demonstrated that it is often wiser to offer each volunteer a brief outline of the study's design, especially when he is to repeat the same experiment with each of a number of preparations. As a rule, this engenders a feeling of participation in the study that enhances the subject's diligence in following the instructions given to him.

The projects suitable for investigation in industrial medical departments may be classified into the following broad categories:

1. Studies of drugs used in the relief of symptoms or minor ailments not usually encountered in hospital or clinic practice.
2. Studies of drugs used in the treatment of ambulatory cases of chronic or recurrent disorders.
3. Studies of the effects of drugs on "normal" individuals.

All may involve the evaluation of a single dose or of a more or less prolonged course of treatment. Not only may the subjective effects of drugs be observed, but their in-

fluence on functional capacities may be demonstrated by measurements made as the subject performs his usual job in his normal work environment.

Nonoccupational illnesses are responsible for the largest portion of lost productivity attributable to medical causes—about fifteen to one hundred times that owing to all injuries and diseases of occupational origin. Because of their great frequency, much of this loss can be charged to such relatively benign conditions as the common cold, gastrointestinal upsets, menstrual disorders, headaches, and musculoskeletal difficulties. Although ethical and practical considerations preclude undertaking the definitive diagnosis and treatment of nonoccupational diseases, the industrial physician does have the responsibility of administering first aid and symptomatic relief to workers troubled by them. He attempts to identify those individuals with more serious or recurrent illnesses so that they may be referred to their private physicians as promptly as possible. The remainder, most of whom would not receive professional medical attention but for the convenience and accessibility of the industrial medical department, are treated to minimize the deleterious effects of their symptoms on their productive capacity. Most of the pharmacologic studies conducted in industrial medical departments attempt to measure the effectiveness of drugs in such cases. Subjects for these studies virtually select themselves as they come to the medical department for alleviation of the particular illness or complaint. It is necessary only to verify the diagnosis and to exclude those persons with complicating conditions that may obscure the drug action being evaluated and those likely to be careless or uncooperative in noting and reporting their responses. The remainder may be offered the experimental drug and instructed both in its use and the procedures to be used in noting its effects. The industrial physician should not allow his desire for a "pure" experiment to outweigh his primary responsibility to restore the normal functional capacities of the ailing employee by relieving his distress with reasonable promptness and safety. Proper design of the study, however, will usually obviate the possibility of conflict on this score.

A slightly different approach is required in recruiting subjects for studies of drugs employed in the treatment of chronic or recurrent disorders. Many of these individuals will already be following regimens prescribed by their personal physicians or will be using proprietary medications, often with excellent results. Consequently, instead of waiting for them to come to him for symptomatic relief as in acute illnesses, the industrial physician will usually have to send for them. His familiarity with the medical status of his employee population derived through routine examinations, previous requests for treatment, and the record of absences owing to medical causes usually enables him to identify them readily. The use of a code for marking the individual case records in his files according to diagnosis or, better still, a separate diagnostic file will make this task quite easy. A review of the individual records will enable him to weed out those with complications likely to interfere with the necessary observations, those who might be too critically balanced in terms of either health status or job assignment to risk interrupting their established regimens, and those who might be undesirable subjects for any other reasons.

Whenever possible, the personal physician of an apparently suitable subject should be consulted before any attempt is made to recruit him. The response usually reflects that physician's attitudes toward industrial medicine more than his concern about any possible effects on his patient's condition. This emphasizes the fact that clinical research should only be undertaken in an industrial medical department with a firmly established occupational health program of high quality in which effective collaboration with employees' personal physicians is successfully fostered. In keeping with this principle, the subjects should be

referred back to their private physicians at the completion of the study, with detailed information about their responses to the drugs being investigated and any changes in their condition noted during the study. Otherwise, there is likely to be a gap between the end of the experimental observations and the resumption of a suitable therapeutic regimen during which their condition may deteriorate unnecessarily.

The types of studies described above both involve the evaluation of drugs in the treatment of the subject's symptoms or the disorder causing them. An altogether different situation is encountered when one desires to study the effects of drugs in "normal" individuals. Here, a presumably healthy individual who needs no medication is to be given drugs with the implicit possibility of a deleterious effect. There will be some loss of time necessitated by his participation in the study and possibly, also, a drug-induced impairment of his job performance. Volunteers for such studies are usually recruited among university students, military personnel, or inmates of prisons. In many instances, a modest fee is an inducement for volunteering. In others, it may be the hope of special consideration from the appropriate authorities,, the prospect of avoiding unpleasant assignments, or simply a change from a boring and monotonous daily routine.

None of these are applicable, as a rule, when recruiting among industrial employees. The payment of money to volunteers, however small the amount, can sometimes create serious difficulties. In some plants, for example, the privilege of earning any extra pay is determined by considerations such as seniority, usually defined in minute detail in the labor-management contract. It may be difficult for a would-be volunteer to accept rejection because he does not fit the criteria created in the protocol when he feels that he is being unfairly deprived of a chance to make some "easy" extra money. By the same token, it would be unwise to allow any hint that volunteers would receive preferential treatment of any kind. It is most important to provide convincing reassurance that those who reject the invitation to volunteer or fail to complete the study, regardless of the reason, will neither lose favor nor be subject to any discrimination in the medical department or elsewhere in the company.

Even when the direct costs are met by research grants, management makes a substantial contribution by allowing a study to be performed in the medical department. When translated into dollars according to prevailing wage scales, the man-hours devoted to a study by the employee volunteers usually represent an impressive amount. In addition to any loss of work efficiency induced by the experimental drugs, there is always the possibility of the kinds of labor-management stress mentioned earlier. It should be obvious that the performance of such a study is indicative of the high regard of management for the caliber of its health program and the capabilities of its medical personnel.

### Launching an experiment

The problems in launching a study of this type are illustrated by a recent experience in clinical psychopharmacology.[1] It involved a double blind comparison of the changes in mood and work performance induced by small single doses of three drugs and a placebo. A battery of tests measuring mood and work performance was administered just before and 2 hours after the ingestion of one of the coded tablets, the subject returning to his usual work during the interval. The four experiments were performed at weekly intervals, if possible on the same day and at the same time.

The subjects for these experiments were recruited from among the employees who happened to visit the medical department on a particular day. Individuals with chronic disease, those with emotional symptoms blatant enough to justify the clinical diagnosis of a neurosis, and those who regularly or frequently took any medication likely to affect the central nervous system were excluded from the study. Those

whose work required extremely irregular activity or much traveling were also excluded. With these exceptions, the subjects were recruited in the order of their appearance without regard to age, sex, position, or kind of work, until all of the vacancies in the schedule of experiments were filled. Executives, department heads, supervisors, and rank and file employees were all represented in the subject population.

Each potential subject was informed of the general purpose of the study and the procedure to be employed. The double blind technique was described and the subject was assured that, although the exact nature of the medication to be used in each experiment would be "unknown," the drugs to be employed were well known and of established safety. He was told that while stimulant or depressant effects might be noted after one or another of the tablets, these would be mild and of brief duration. Finally, he was assured that no penalties would be assessed for refusal to participate or for failure to complete the series of experiments, neither would any explanation be required for either action.

The study required that the subjects be "normal" at the time of each experiment. Consequently, an experiment was postponed whenever a subject reported or appeared to manifest any form of intercurrent illness or any unusual emotional stress or excitement. Furthermore, while the experiments were originally scheduled at a time calculated to interfere least with his work assignments on the basis of consultation with both the subject and his supervisor, they were also postponed if either indicated an unexpected degree of job pressure, whatever the cause.

The instructions for the scheduling of the experiments which follow demonstrate the difficulty of conducting this kind of study with minimum cost to management.

***Preliminary arrangements.***

1. On acceptance of the invitation to participate, the subject is tentatively scheduled for four experiments, one each week on the same day and at the same time.

2. Approval of the subject's participation is obtained from his supervisor or department head, who also verifies the convenience of the tentative schedule.

3. A memorandum confirming the schedule is sent to both the subject and his supervisor.

***Steps on the day of the experiment.***

1. Telephone the supervisor to confirm the fact that the work schedule will permit the subject to be excused to perform the experiment at the appointed time.

2. Telephone the subject to remind him or her of the scheduled appointment and to warn against changes in lunch schedule and partaking of coffee or snacks that might interfere with the experiment.

3. If either the subject or his supervisor indicates that the appointment presents difficulties, cancel it and reschedule for any alternative time available on the master appointment schedule.

4. Be sure that the appropriate test materials, record forms, and the drug envelope assigned for the particular experiment are set aside and ready to be used.

While no record was made of the number of telephone calls, memoranda, or the man-hours of staff time involved in this

**Table I.** *Results of recruiting interviews for experiments on effects of drugs on mood and work performance in industry*

| *Status* | *Number of subjects* |
|---|---|
| Completed full series of experiments | 55* |
| Started but dropped out before completion | 1 |
| Agreed to volunteer but not used because of termination of study | 5 |
| Agreed to volunteer but "changed mind" before starting experiments | 7 |
| Agreed to volunteer but unable to participate because of work conflicts, etc. | 16 |
| Suitable for study but refused to participate | 39 |
| Total interviewed | 123 |

*Twenty-eight subjects completed a second series of experiments.

endeavor, the time and effort expended were unquestionably significant, probably far more than would be required for a similar study in a population of students, clinic patients, or paid volunteers. However, this investment appears to have been rewarded by the fact that only one subject failed to complete his full series of experiments (Table I). Since, according to the research plan, no data could be used unless the subject completed all of his assigned experiments, the saving of investigator-subject time afforded by such a low incidence of dropout was also considerable. Furthermore, the concerted effort to avoid undue interference with the subject's work assignments made it much easier to obtain management's permission for the continuation of such investigations.

## Conclusions

This study amply demonstrates the feasibility of conducting research in clinical pharmacology in the setting of industrial medicine. The generous cooperation of the managements of the three companies participating in this project* and their contributions of the use of facilities and the considerable total of employee man-hours required to perform these experiments attest to the willingness of industry to permit and to assist such scientific endeavors. The eagerness of all ranks and categories of employees to serve as volunteers, without remuneration and without preferential treatment, and the endorsement of their participation by some of their labor organizations confirm the ready availability of subject populations suitable for this kind of investigation. The solutions of some of the special problems involved in recruiting subjects and in planning and performing the experiments illustrate the effort that must be made to fit such research activities into the framework of the daily practice of modern industrial medicine. Finally, although mentioned only tangentially, the necessity of strict adherence to satisfactory standards of scientific accuracy and objectivity is reiterated.

The current flood of newly discovered and synthesized drugs demands an expansion of the clinical research activities aimed at measuring their therapeutic value and exploring their other effects in humans. Unfortunately, changes in the socioeconomic aspects of medical care, among other factors, have produced both a shrinkage in the patient populations from which suitable subjects have traditionally been obtained and a relative lack of the medical and technical personnel capable of performing such investigations. Industrial medical departments can supply these deficiencies. In addition, they offer opportunities to the industrial physician trained in clinical pharmacology that are difficult if not impossible to duplicate in hospital or outpatient practice. Among these are his ability to control the environment for the duration of the effect of a drug, to demonstrate the effects of drugs on functional capacities by measuring the changes in the performance of routine work assignments, and to make repeated and prolonged observations without interfering with the subject's opportunity to earn his income. There seems little doubt, therefore, that industrial medical departments will serve increasingly as arenas for research in clinical pharmacology.

### *Reference*

1. Warshaw, L. J.: The effects of drugs on mood and work performance in industry. In preparation.

*United Artists Corp., Paramount Pictures Corp., and American Broadcasting–Paramount Theatres, Inc.

# Symposium on the experimental pharmacology and clinical use of antimetabolites

## Part VI. Mechanisms of resistance to metabolite analogues with anticancer activity

**R. W. Brockman, Ph.D.** *Birmingham, Ala.*
*Kettering-Meyer Laboratory,* Biochemistry Division, Southern Research Institute*

It may be said that drug resistance has followed chemotherapy like a shadow; Ehrlich and his colleagues recognized and described this phenomenon in one of the first examples of chemotherapy, the treatment of trypanosomiasis in mice.[2] The problem of drug resistance in chemotherapeutic treatment of bacterial infections in man has been widely recognized since the discovery of sulfa drugs and antibiotics.[87]

The discovery of drugs that have temporary anticancer activity, particularly in certain forms of leukemia, was rapidly followed by recognition of the development of drug resistance in neoplasms. Burchenal and associates[34] and Law and Boyle[101] first observed resistance to amethopterin in experimental neoplasms, and Law[99] has stated that "the problem of resistance in neoplastic cells, as in microorganisms, will remain a most important, perpetual threat to the use of therapeutic agents." Burchenal[31] and Karnofsky[92] have cited the invariable development of resistance as a cause of failure of therapeutic agents in clinical cancers that are initially responsive to drug treatment—particularly in leukemia.

During recent years, the problem of drug resistance to anticancer agents has been approached from a biochemical standpoint. The questions asked are: (1) Are alterations in the metabolism of cells coincident with the development of heritable resistance to anticancer drugs? (2) What is the biochemical basis for such alterations when they are observed to occur? (3) How can such drug resistance be circumvented? In the case of certain purine and pyrimidine analogues known to possess anticancer activity, such as 6-mercaptopurine and 5-fluorouracil, it is now possible to propose rather specific answers to the first two questions. It was observed that resistance to these drugs in microorganisms and in mouse neoplasms was accompanied by decreased capacity of the resistant cells to metabolize the analogues to ribonucleotides as compared with the parent drug-sensitive lines. Studies with enzyme preparations have shown that resistant microbial and neoplastic cells have a decreased capacity to catalyze the conversion of certain of the purine and pyrimidine analogues to

The work on drug resistance in this laboratory is supported by the Cancer Chemotherapy National Service Center of the National Institutes of Health and by grants from the Charles F. Kettering Foundation and the Alfred P. Sloan Foundation.

Received for publication March, 1960.

*Affiliated with Sloan-Kettering Institute for Cancer Research, New York, N. Y.

ribonucleotides. As to circumvention of resistance arising by this mechanism, drug sensitivity could presumably be re-established if capacity to synthesize the fraudulent nucleotide could be restored; this possibility has not yet been explored. If a drug becomes active as a result of metabolism by the cell to a more inhibitory derivative, the product of such a synthesis, when introduced into the cell, should inhibit growth of resistant cells. Synthesis of fraudulent purine and pyrimidine nucleotides and derivatives has become an active field for the efforts of organic chemists.

Drugs such as azaserine and amethopterin, on the other hand, appear to be inhibitory as such and do not require metabolic activation by the cell. It appears that resistance to such inhibitors may arise as a result of decreased affinity of specific enzymes for the inhibitors or decreased access of the inhibitors to the sensitive enzymes. At present, the most promising approach to the third question posed—circumvention of resistance—appears to be through use of combinations of inhibitors. The possibilities of combined therapy were recognized by Ehrlich[51] many years ago.

This paper presents a brief discussion of recent studies on mechanisms of resistance to compounds some of which are known to inhibit nucleic acid synthesis (Fig. 1). An effort has been made to present some of the historical background to illustrate the growth of the present body of literature referred to in recent extensive reviews. It is neither possible nor desirable to separate the double-edged problems of mechanisms of drug inhibition and mechanisms of drug resistance, for advances gained in one area usually contribute to understanding in the other. Detailed knowledge of the biosynthesis of nucleic acids has engaged the best efforts of biochemists, and results of these studies have made possible a clearer understanding of the mechanism of action of some of the inhibitors under discussion. In return, the biochemist is indebted to those workers who discovered inhibitors such as amethopterin and azaserine—agents which have proved to be valuable tools in studying pathways of metabolism.

The author has found invaluable the following recent reviews: Anderson and Law on the biochemistry of cancer,[5] Buchanan and Hartman on purine biosynthesis,[30] Handschumacher and Welch on agents which influence nucleic acid metabolism,[67] Mandel on the physiologic disposition of anticancer agents,[111] Matthews on biosynthetic incorporation of metabolite antagonists,[116] Reichard on pyrimidine biosynthesis,[134] and Welch on drug resistance in cancer chemotherapy.[160]

### Concepts of lethal synthesis and of enzyme deletion

These concepts are introduced for consideration because, when taken together, they offer the simplest interpretation of one mechanism of resistance to purine and pyrimidine analogues observed in certain biologic systems.

Liebicq and Peters[106] and Martius[115] independently discovered that $\alpha$-fluoroacetic acid was metabolized to fluorocitric acid and that this latter substance was a powerful inhibitor of the enzymic conversion of citric acid to aconitic acid, thus wrecking the important Krebs cycle. Peters[129] termed this conversion of fluoroacetic acid to fluorocitric acid by the same pathway that acetic acid is normally metabolized a "lethal synthesis." Other familiar examples of the conversion of less toxic to more toxic substances can be cited. A classic example is the observation that *p*-aminophenylarsonic acid (atoxyl) was not inhibitory to trypanosomes in vitro[156] but was inhibitory in vivo.[49] Ehrlich's investigation of this difference led to the finding that the pentavalent arsonic acid was noninhibitory and that reduction in vivo to the trivalent arsenoxide yielded an active inhibitor.* Following this lead, Ehrlich[50] launched the work that led to the discovery of the first clinically useful chemotherapeutic agent,

*These conversions do not necessarily take place within the microorganisms; prontosil, for example, is reduced to sulfanilamide in the blood stream of the host.

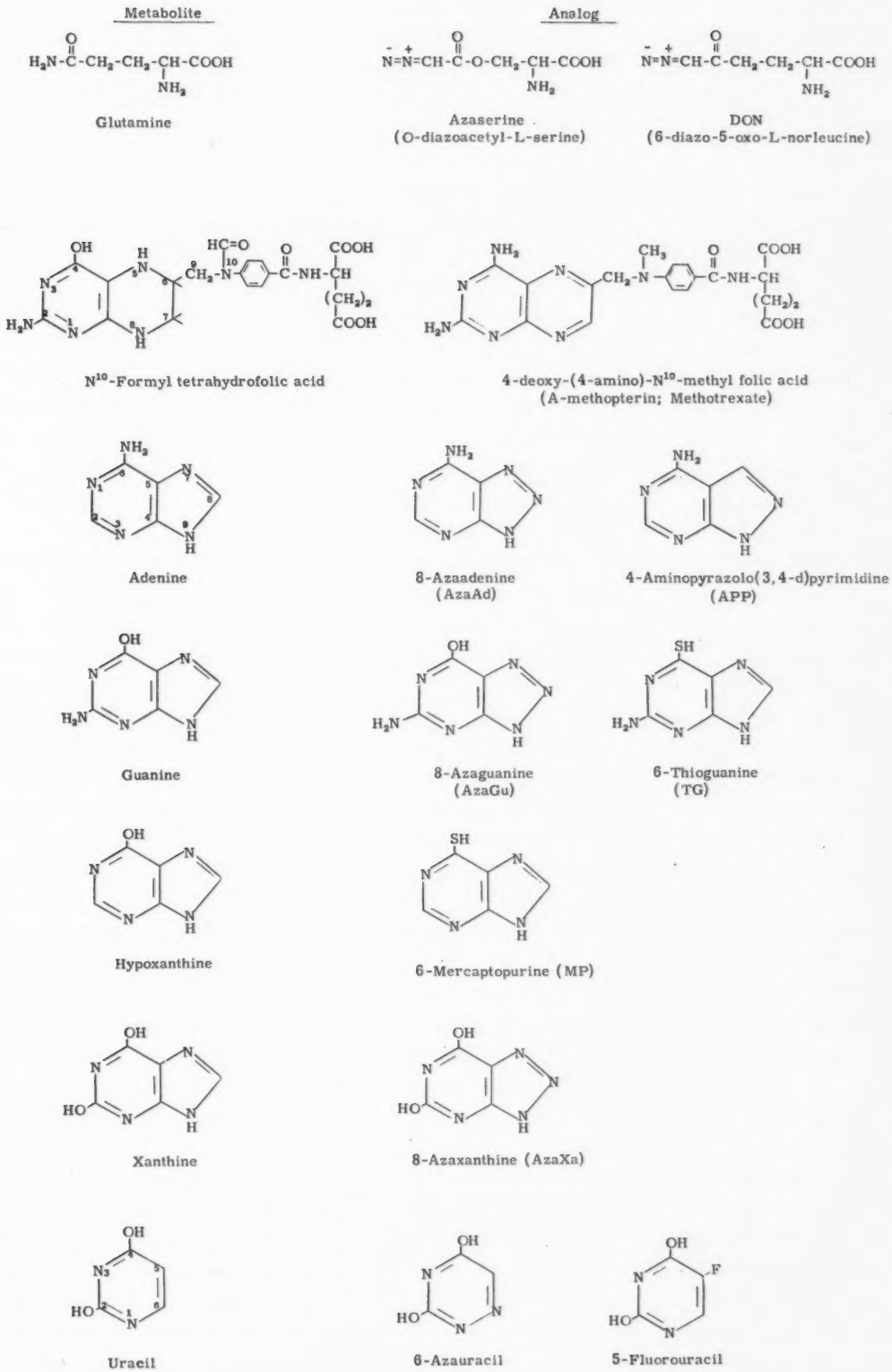



**Fig. 1.** Structures of metabolite analogues. Amethopterin and azaserine are inhibitory as such and are known to inhibit biosynthesis of inosinic acid. It appears likely that the other analogues listed become inhibitory by conversion to nucleotides (see Fig. 3).

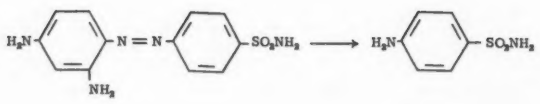



**Fig. 2.** Activation of prontosil by reduction to sulfanilamide.

arsphenamine*—an arsenobenzene derivative which becomes active after oxidation to the arsenoxide. Another example can be drawn from work on the sulfa drugs. Domagk[48] and his associates discovered prontosil to be an effective antibacterial agent. Tréfouel, Nitti, and Bovet[155] found that sulfanilamide, which is derived from prontosil (Fig. 2), was an effective antibacterial agent; prontosil does not act until it is reductively cleaved to sulfanilamide.

In the results to be discussed in detail below it will be seen that the purine analogues 8-azaguanine, 6-mercaptopurine, and 6-thioguanine are metabolized to ribonucleotides by sensitive bacterial and neoplastic cells. These fraudulent ribonucleotides are thought to be the active inhibitors, and their formation can thus be viewed as a lethal synthesis (Fig. 3). As a result of the extensive research of Heidelberger and associates,[16, 35, 36, 43, 68, 71-76, 123] it has been found that 5-fluorouracil is converted to 5-fluorouridylic acid and then to 5-fluoro-2′-deoxyuridylic acid, an irreversible inhibitor of thymidylic acid synthesis.[41] This too is viewed as a lethal synthesis.

In studies of resistance to 8-azaguanine, 6-mercaptopurine, 6-thioguanine, 6-azauracil, and 5-fluorouracil, it has been found that certain drug-resistant cells have lost the capacity to accomplish the formation of nucleotides of these purine and pyrimidine analogues. As a result of decreases or losses of specific enzyme capacities, the resistant cells fail to carry out the lethal synthesis of fraudulent nucleotides. Studies on the biochemical effects of mutations have shown that gene alteration can lead to the disappearance of specific enzyme activities of the cell. The classic work of Beadle[13] established the gene-enzyme relationship through detailed studies of x-ray-induced mutants of the mold *Neurospora crassa.* For example, Beadle was able to show that auxotrophic mutants of this organism had suffered loss of specific enzymes and were thus unable to grow unless the appropriate product formed by the lost enzyme was added to the growth medium. Illustrations of genetically controlled enzyme deletions in humans can be drawn from what Garrod termed "inborn errors of metabolism."[60] One example of an inborn error, congenital galactosemia, is now known to be a consequence of enzyme deletion from the recent demonstration that the enzyme which catalyzes the reaction

$$\text{Gal-1-PO}_4 + \text{UDPG} \xrightarrow{\text{Enzyme}} \text{G-1-PO}_4 + \text{UDPGal}^*$$

is missing in red blood cells and in liver of the galactosemic individual.[4, 89] The uridine diphosphate galactose thus formed is isomerized by a second enzyme to uridine diphosphate glucose. Without the reaction depicted above, this sequence of events cannot take place and the galactose ingested by the galactosemic infant accumulates. Numerous examples of enzyme deletion can now be defined in biologic systems as diverse as microorganisms and humans.[57, 80]

Heritable resistance to certain purine and pyrimidine analogues appears to arise in some bacteria and experimental neoplasms by genetically controlled alteration of specific enzymes which catalyze formation of nucleotide derivatives of these analogues. Such alterations are sometimes so drastic that enzyme activity is lost. Random mutation, which could result in enzyme deletion, followed by selection in the presence of drugs, appears to be one important mechanism by which drug-resistant cells can arise. Specific examples and extensions of

*Salvarsan.

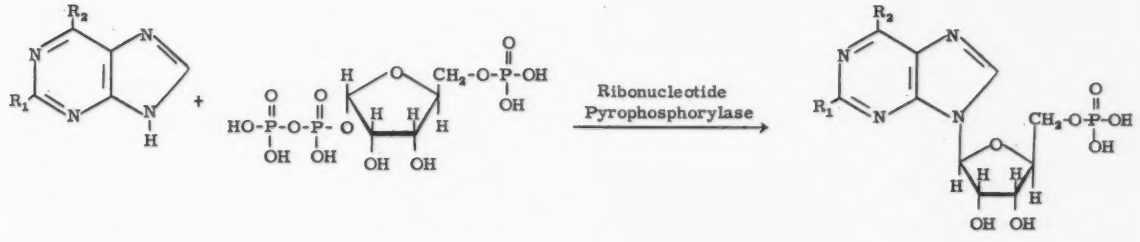
*Gal-1-PO$_4$, galactose-1-phosphate; UDPG, uridine diphosphate glucose; G-1-PO$_4$, glucose-1-phosphate; UDPGal, uridine diphosphate galactose.

these ideas are presented below. Enzymatic mechanisms of resistance to azaserine and amethopterin have not yet been clearly defined.

### Resistance to purine analogues

***8-Azaguanine.*** The interesting history of the first purine analogue shown to inhibit experimental neoplasms in mice began with the synthesis of a group of 8-azapurines (see Fig. 1) by Roblin and his associates[136] in 1945. Kidder and his group[93] at Amherst noted the profound inhibitory effect that 8-azaguanine had on the growth of certain protozoa and therefore examined this compound for its effect on growth of mouse neoplasms. It was then shown that 8-azaguanine was incorporated into nucleic acids of mouse neoplasms.[119] Since that time, 8-azaguanine as well as other purine and pyrimidine analogues has been observed to be incorporated into the nucleic acids of viruses, bacterial cells, protozoa, neoplastic cells, and nonneoplastic cells.[116]

Earlier studies in this laboratory demonstrated that 8-azaguanine-resistant mouse leukemia cells incorporated less 8-azaguanine-2-$C^{14}$ into their nucleic acids than did the drug-sensitive leukemia cells.[15] Way and Parks[158, 159] showed that 8-azaguanine, 6-mercaptopurine, and other purine analogues underwent reaction with 5-phosphoribosylpyrophosphate to yield nucleotide derivatives just as did natural purines. Lukens and Herrington[108] also demonstrated enzymic formation of 6-mercaptopurine ribonucleotide. At the time these observations were reported, we were engaged in a study of the purine metabolism of bacteria sensitive or resistant to growth inhibition by purine analogues and observed that resistant bacteria were unable to convert certain of the purines and purine analogues to ribonucleotides.[18, 26] This result fitted well with earlier observations of Hutchison[82] and Elion, Singer, and Hitchings[54, 55] which showed that such resistant bacteria had lost capacity to utilize certain of the purines for growth. It was apparent that 8-azaguanine-resistant and 6-mercaptopurine-resistant bacteria had lost the capacity to convert these purine analogues to the corresponding ribonucleotides and, as a consequence of this, had also lost the capacity to incorporate 8-azaguanine into their nucleic acids[21, 25] (Table I). Hutchison[82] had previously observed that 6-mercaptopurine-resistant bacteria (designated SF/MP in Table I) were cross-resistant to 8-azaguanine but were still sensitive to 8-azaxanthine and that this mutant was still able to utilize xanthine for growth. Similarily, growth of the 8-azaguanine-resistant bacteria (SF/AZAG) was inhibited by 8-azaxanthine, just as was the parent strain of *Streptococcus faecalis* (SF/O). A study[25] of the metabolism of 8-azaxanthine-2-$C^{14}$ revealed that this inhibitor was converted to 8-azaguanylic acid-2-$C^{14}$ by those strains of bacteria that were sensitive to this analogue. This can best be illustrated by reference to Fig. 4*B*; 8-azaxanthine is evidently converted to 8-azaxanthylic acid and then to




Purine base (or analog) + 5-phosphoribosyl-1-pyrophosphate $\xrightarrow{\text{Enzyme}}$ Purine ribonucleotide + pyrophosphate

Fig. 3. Biosynthesis of purine ribonucleotides.

**Table I.** *Metabolism of 8-azaguanine-2-C$^{14}$ and 8-azaxanthine-2-C$^{14}$ by drug-sensitive and drug-resistant Streptococcus faecalis*

| *Event studied* | *SF/O* | *SF/AZAG* | *SF/8-Aza* | *SF/MP* |
|---|---|---|---|---|
| *Sensitivity of microorganisms to growth inhibition by* | | | | |
| 8-Azaguanine | S | R | R | R |
| 8-Azaxanthine | S | S | R | S |
| *Percentage of soluble fraction present as 8-azaguanosine nucleotides* | | | | |
| 8-Azaguanine | 68 | 0 | <4 | <5 |
| 8-Azaguanosine mono-, di-, and triphosphates | | | | |
| 8-Azaxanthine | 37 | 20 | 0 | 35 |
| *Specific activity of nucleic acid (counts per second per 500 μg)* | | | | |
| 8-Azaguanine | 1,235 | 7 | 1 | 26 |
| Incorporation into nucleic acids as 8-azaguanylic acid | | | | |
| 8-Azaxanthine | 492 | 225 | <1 | 200 |

S indicates sensitivity and R designates resistance to the inhibitors.

SF/O, parent drug–sensitive S. *faecalis;* SF/MP, resistant to 6-mercaptopurine (and 8-azaguanine); SF/AZAG, resistant to 8-azaguanine (and 6-mercaptopurine); SF/8-Aza, resistant to 8-azaguanine, 8-azaxanthine, and 6-mercaptopurine.

8-azaguanylic acid, which can then be incorporated into nucleic acids. Here, then, is an example of circumvention of resistance by formation of the active form of the inhibitor by another pathway. Still another bacterial mutant was obtained which was resistant to both 8-azaguanine *and* 8-azaxanthine (SF/8-Aza). This mutant was found to have lost the capacity to metabolize 8-azaxanthine to 8-axaguanylic acid and to incorporate radioactivity from 8-azaxanthine-2-C$^{14}$ into nucleic acid.

How did this loss of capacity to metabolize certain of the purines and 8-azapurines come about? Was it a result of a decrease in permeability of resistant cells to these compounds or was it a consequence of an intracellular biochemical alteration? Davidson[44] ruled out decreased cell permeability as a mechanism of resistance to certain of these purine analogues in mouse leukemia. We elected then to turn attention to a comparison of the capacity of enzyme preparations from drug-sensitive and drug-resistant cells to catalyze the reaction (Fig. 3) discovered in the laboratories of Kornberg[95] and of Buchanan.[94] Results of these studies clearly showed that enzymes isolated from 8-azaguanine-resistant and 6-mercaptopurine-resistant S. *faecalis* had significantly less capacity to catalyze the formation of 8-azaguanylic acid and 6-mercaptopurine ribonucleotide. In some bacterial mutants (SF/MP and SF/AZAG) this capacity appeared to be lost, and in others it was significantly decreased.[20, 28] Salser and associates have also observed this decreased capacity of 6-mercaptopurine-resistant bacteria to form the nucleotide.[137, 138]

Thus, using bacteria as a model system, it was possible to establish the fact that resistance to 8-azaguanine and 6-mercaptopurine was clearly associated with the markedly decreased capacity of resistant cells to form the nucleotide (enzyme dele-

ion or inactivation); conversely, sensitivity of these bacteria to these same purine analogues was clearly associated with formation of the fraudulent nucleotide (lethal synthesis).

The use of bacteria as a model system for studying metabolic pathways is of established value. The comments of Fizz-Looney and Linderstrøm-Lang[58] on the use of model systems suggest the advisability of pursuing leads gained from the study of simple biologic systems in more complex systems whenever possible:

"One well established and generally accepted method of treating systems which are complicated beyond comprehension is to construct simple models and see whether they fit the systems in question. If they do, you will immediately become suspicious, and so will your colleagues most certainly, with the result that a blooming literature springs up (or breaks out) dealing with the problem of how you have managed to make all your errors cancel one another. If they do not fit, the beauty of the models themselves may shine for years untainted by the squalid awkwardness of reality."

The observations in bacterial model systems have been extended to studies on resistance to purine analogues in transplantable mouse neoplasms—yet another model system. The results obtained in a study of the capacity of a spectrum of mouse neoplasms to form 8-azaguanine ribonucleotides from 8-azaguanine-2-$C^{14}$ in vivo have shown that neoplasms that are inhibited by 8-azaguanine form significant amounts of 8-azaguanine ribonucleotides and incorporate 8-azaguanylic acid into their nucleic acid, whereas neoplasms that are resistant to 8-azaguanine fail to form significant amounts of 8-azaguanine ribonucleotides and, consequently, do not incorporate the analogue into their nucleic acids. The data summarized in Fig. 5 reveal this very clearly.[17] Results of enzyme studies showed that resistant neoplasms, like resistant bacteria, had lost significant capacity to form fraudulent nucleotides via the reaction depicted in Fig. 3.[17, 28]

Szybalski[151] has recently isolated 8-azaguanine-resistant lines of human bone marrow cells grown in tissue culture. Preliminary results of a collaborative study in this laboratory comparing the metabolism of guanine-8-$C^{14}$ and 8-azaguanine-2-$C^{14}$ indicate that these resistant cell lines also have a decreased capacity to form nucleotide derivatives from the bases.

Mandel showed that 4-amino-5-imidazolecarboxamide (AIC) inhibited deamination of 8-azaguanine to 8-azaxanthine,[112] a degradative product devoid of anticancer activity in mouse neoplasms. As the results summarized in Fig. 5 show, AIC increased the incorporation of 8-azaguanine into 8-azaguanylic acid and into nucleic acids of drug-sensitive L1210, but not into nucleic acids of drug-resistant L1210 leukemia. Thus, inhibition of this degradative reaction in resistant cells did not result in increased formation of 8-azaguanylic acid in resistant cells—a result which supports the view that resistance to 8-azaguanine in this system is a consequence of inability to form the nucleotide and not of increased capacity to degrade 8-azaguanine.

An interesting mechanism of inhibition postulated for 8-azaguanine, or more likely for a nucleotide of 8-azaguanine, in bacterial cells has recently been described by Mandel and associates.[110, 113, 114] Results of these studies have shown that cells grown in the presence of 8-azaguanine synthesized protein molecules with altered composition—particularly with regard to sulfur-containing amino acids. It may be that such altered protein synthesis is a consequence of the incorporation of 8-azaguanine into ribonucleic acid or of an inhibitory effect produced by an 8-azaguanine ribonucleotide.

An interesting facet of the study of resistance to azapurines is the demonstration by means of different resistant bacterial mutants of at least three distinct enzymes catalyzing the formation of ribonucleotides of purines[24] (see Fig. 4). For example, mutants resistant to 8-azaguanine and 6-mercaptopurine have lost the capacity to form

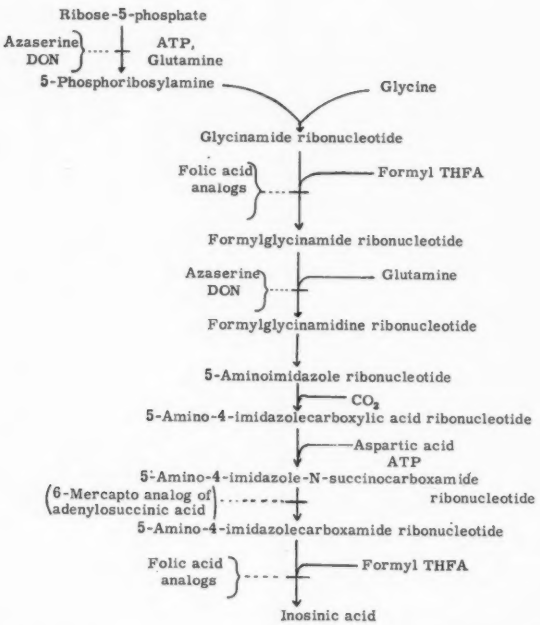



**Fig. 4A.** Diagram of de novo biosynthesis of inosinic acid (see references 30 and 69), showing known sites of inhibition of azaserine, DON, and folic acid analogues.

inosinic and guanylic acids from the corresponding bases but retain capacity to form adenylic and xanthylic acids from adenine and xanthine, respectively, by the reaction shown in Fig. 3. A different mutant, resistant to 8-azaxanthine, has lost capacity to form xanthylic and 8-azaxanthylic acids but retains capacity to form all other nucleotides. Still other mutants resistant to 8-azaadenine and 2-fluoroadenine have lost capacity to form adenylic acid but retain capacity to form xanthylic acid. The interpretation placed on these data is that (1) specific genes control the formation of the enzymes which catalyze these reactions, (2) resistance to a given purine analogue arises by mutation (probably stepwise), and (3) selection in the presence of high concentrations of inhibitor results in survival of those cells which have suffered significant loss of enzyme capacity to form the nucleotide of the particular purine an-

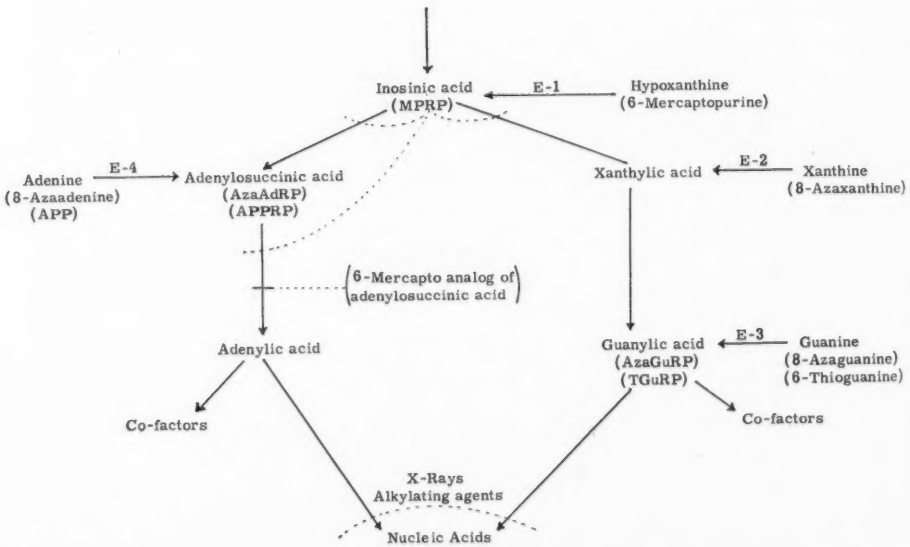



**Fig. 4B.** Diagram of interconversions of purine ribonucleotides. The ribonucleotide pyrophosphorylases which catalyze the reactions of natural purines with 5-phosphoribosyl-1-pyrophosphate to yield adenylic, inosinic, xanthylic, and guanylic acids also catalyze formation of purine analogue ribonucleotides. There is evidence suggesting that in some biologic systems inosinic acid pyrophosphorylase *(E-1)* and guanylic acid pyrophosphorylase *(E-3)* may be identical.[24] *APP*, 4-aminopyrazolo(3,4-d)pyrimidine; *APPRP*, corresponding ribonucleotide; *MPRP*, 6-mercaptopurine ribonucleotide; *AzaAdRP*, 8-azaadenylic acid; *AzaGuRP*, 8-azaguanylic acid. The sites of inhibition indicated for 6-mercaptopurine ribonucleotide have been demonstrated in vitro and in isolated enzyme systems[46, 65, 138]; x-irradiation and alkylating agents appear to inhibit nucleic acid synthesis at a later stage.

alogue. Fig. 4 depicts the conversion of natural purines and analogues to nucleotides by these different enzymes. Since capacity to form inosinic and guanylic acids seems to be lost simultaneously in bacterial and neoplastic cells resistant to 6-mercaptopurine and 8-azaguanine, it may be that the same enzyme (or enzymes closely linked genetically) catalyzes these reactions.

***6-Mercaptopurine.*** This purine analogue was synthesized by Elion, Burgi, and Hitchings[53] and first observed to have anticancer activity by Clarke and co-authors[38] and by Law.[98] Since that time, much experimental and clinical research has been done with 6-mercaptopurine,[118] and design of other analogues of purines has been an active area for synthesis of candidate anticancer agents.[37, 120, 145]

It was observed that 6-mercaptopurine inhibited the labeling of nucleic acid purines of neoplasms by formate-$C^{14}$ [77, 142] and by glycine-$C^{14}$.[103] In recent studies, Salser and associates,[137, 138] using bacterial enzyme preparations, found that synthetic 6-mercaptopurine ribonucleotide was inhibitory to the formation of adenylsuccinic acid from inosinic acid, and Davidson has shown that 6-mercaptopurine, probably as the nucleotide, inhibits the synthesis of adenylic acid from inosinic acid in L1210 cells[45, 46] (see Fig. 4*B*). There appear to be other sites of inhibition by nucleotide derivatives of 6-mercaptopurine, such as the inhibition of conversion of inosinic acid to xanthylic acid,[138] the inhibition of conversion of adenylsuccinic acid to adenylic acid,[65] and the inhibition of the formation of 5-amino-4-imidazolecarboxamide ribonucleotide[117] (see Fig. 4*A*).

Studies of the utilization of exogenous carbon[14]-labeled purines for synthesis of nucleic acids revealed that 6-mercaptopurine-resistant microorganisms utilized guanine and hypoxanthine poorly.[10] The resistant organisms were unable to utilize these purines for growth in a medium in which preformed purines were essential,[82, 84] and exponentially growing 6-mercaptopurine-resistant cells were unable to convert guanine, hypoxanthine, and 6-mercaptopurine to ribonucleotides.[18, 25] Subsequent studies revealed that enzyme preparations from drug-resistant cells were unable to catalyze the formation of ribonucleotide derivatives of guanine, hypoxanthine, or 6-mercaptopurine[28] or had significantly less capacity than sensitive cell preparations to catalyze these reactions.[138*]

Studies of 6-mercaptopurine metabolism have also shown that it is converted to 6-mercaptopurine ribonucleotide by drug-sensitive neoplasms[23, 128] but not by 6-mercaptopurine-resistant neoplasms.[23] It is evident from the results summarized in Fig. 6 that nonneoplastic cells also form 6-mercaptopurine ribonucleotide. Studies with enzyme preparations revealed a decreased capacity for nucleotide formation in the drug-resistant neoplasms.[28] It is of interest that Tomizawa and Aronow[153] recently isolated 6-mercaptopurine-resistant lines of mouse fibroblasts grown in tissue culture and observed that the parent drug-sensitive line formed 6-mercaptopurine ribonucleotide but the resistant line did not.

***6-Thioguanine.*** It was demonstrated that drug-sensitive Ehrlich ascites tumor cells convert the guanine analogue 6-thioguanine to 6-thioguanine ribonucleotides and that these cells incorporate the analogue into nucleic acids.[122] Drug-resistant Ehrlich ascites cells formed less 6-thioguanylic acid and incorporated much less 6-thioguanine into nucleic acids than did drug-sensitive cells.[141] It was also observed that 6-thioguanine-resistant Ehrlich ascites tumor cells had greater capacity to degrade 6-thioguanine than did sensitive cells.[141] Increased degradation of inhibitor is therefore a resistance mechanism to be considered. In studies in this laboratory, such increased degradation of inhibitor does not appear to be a significant factor in resistance to 8-azaguanine,[17] 6-mercaptopurine,[23]

*Balis and co-workers postulated that 6-mercaptopurine might be converted to a nucleotide[12] and that failure to form a nucleotide might be one among several possible mechanisms of resistance to 6-mercaptopurine.[11]

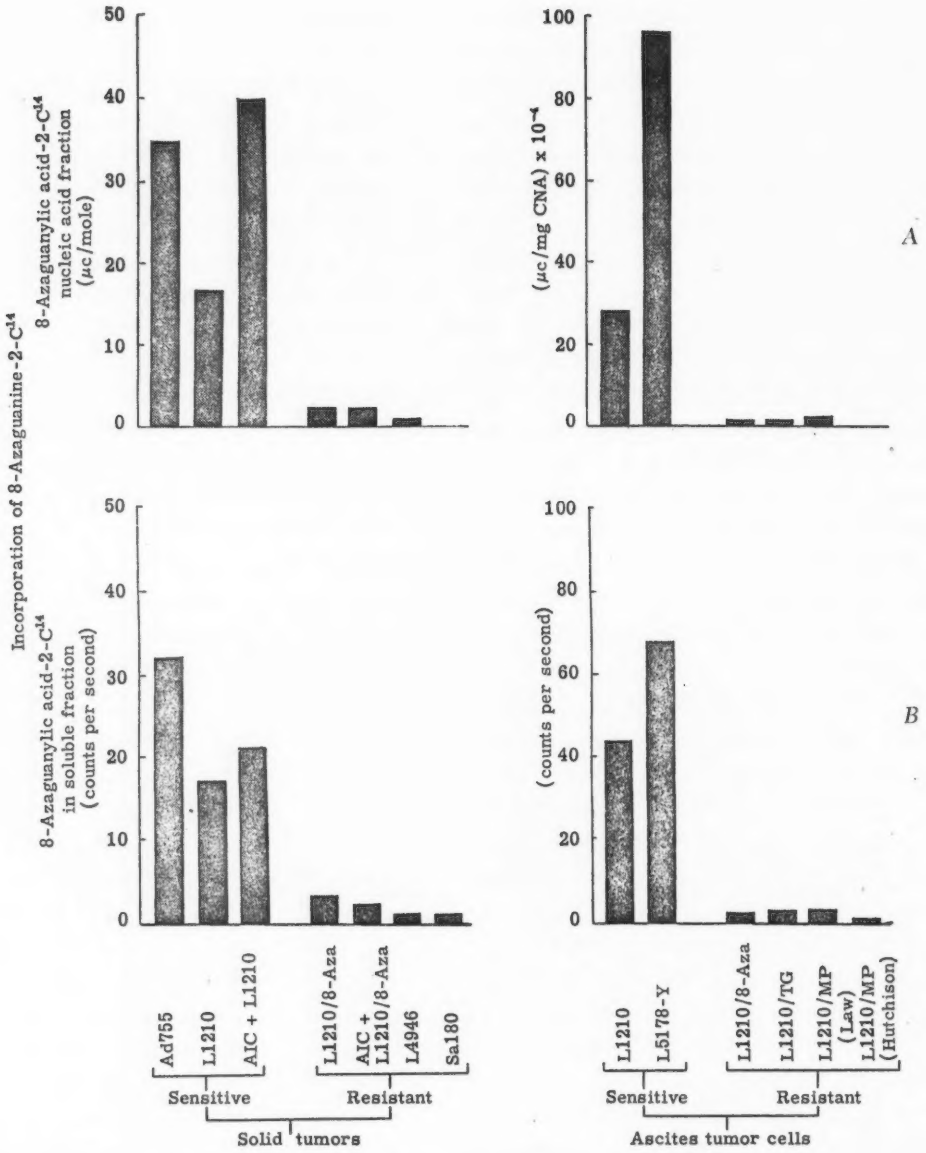



**Fig. 5.** Conversion of 8-azaguanine-2-$C^{14}$ to 8-azaguanine ribonucleotides in vivo by sensitive and resistant neoplasms *(B)* and incorporation of radioactive 8-azaguanylic acid into nucleic acids *(A)*.[17]

*Mouse neoplasms*

*Ad755*, adenocarcinoma; *L5178-Y*, leukemia; *L1210*, leukemia
*L1210/8-Aza*, 8-azaguanine-resistant
*L1210/MP*, 6-mercaptopurine-resistant } Cross-resistant to 8-azaguanine
*L1210/TG*, 6-thioguanine-resistant
*L4946, leukemia*
*Sa180, sarcoma* } "Naturally" resistant to 8-azaguanine

or 6-thioguanine[29] in the resistant L1210 leukemia lines studied. However, significant loss of enzyme capacity to form 6-thioguanylic acid has also been observed in 6-thioguanine- resistant L1210.[29] Sartorelli and LePage,[140] from studies of the effect of 6-thioguanine on nucleic acid precursors, have cited inhibition of conversion of inosinic acid to adenylic acid and inhibition of an early step in denovo purine synthesis.

### Resistance to pyrimidine analogues

Two uracil analogues, 6-azauracil and 5-fluorouracil, will be considered here. There are similarities in the metabolism of these compounds—both are converted to the corresponding ribonucleotide and deoxyribonucleotide derivatives—but the analogues exert their inhibitory effects at quite different sites (Fig. 7).

***6-Azauracil.*** Handschumacher and Welch[67] have recently reviewed the extensive work of the group at Yale on 6-azauracil, and Habermann and Šorm[64] have summarized the independent parallel studies on this drug by the group in Czechoslovakia. From these studies it is evident that 6-azauracil is metabolized to 6-azauridylic acid, which blocks the conversion of orotidylic acid to uridylic acid—a vital step in the de novo synthesis of pyrimidines (Fig. 7). Handschumacher[66] showed that a 6-azauracil-resistant strain of bacteria remained sensitive to inhibition by 6-azauridine but had lost the capacity to utilize uracil-2-$C^{14}$. This mutant thus appeared to have lost the capacity to convert 6-azauracil to 6-azauridylic acid—a reaction which can be viewed as a lethal synthesis.

***5-Fluorouracil.*** From the studies of Harbers, Chaudhuri, and Heidelberger,[68] it is known that 5-fluorouracil is metabolized by the same pathway as is uracil (compare Figs. 7 and 8). It is evident from these studies that 5-fluorouracil is converted to 5-fluorouridine-5′-mono-, di-, and triphosphates, to 5-fluorouridine-5′-disphosphate sugar derivatives, and to 5-fluoro-2′-deoxyuridine-5′-phosphate (5-fluorodeoxyuridylic acid). This latter compound is an inhibitor of thymidylic acid synthesis[41] and hence of deoxyribonucleic acid synthesis. Cohen's acute observations in bacterial mutants on what he has termed "thymine-less death"[40] as a result of unbalanced nucleic acid synthesis* led to consideration of this phenomenon in connection with 5-fluorouracil inhibition.[41] Lindner[107] has observed that 5-fluorouracil inhibition of Ehrlich ascites tumor cells produces a type of abnormal cell compatible with the concept of unbalanced growth.

Duschinsky and his colleagues at Hoffman-LaRoche and Fox and his associates at Sloan-Kettering Institute have synthesized a series of 5-fluoropyrimidine ribonucleosides and deoxyribonucleosides. These include 5-fluorouridine, 5-fluoro-2′-deoxyuridine, 5-fluorocytidine, and 5-fluoro-2′-deoxycytidine; some of these derivatives seem to possess advantages over 5-fluorouracil in cancer chemotherapy. All of these compounds appear to be converted to 5-fluoro-2′-deoxyuridylic acid, and the inhibition of thymidylic acid synthesis is considered to be a key site of inhibition produced by 5-fluoropyrimidines in experimental neoplasms.[43, 68]

The conversion of 5-fluorouracil to 5-fluorouridylic acid is a necessary step in formation of the deoxyribonucleotide and can therefore be viewed as part of a lethal synthesis. In the light of the considerations on resistance to 6-azauracil and to purine analogues, it was logical to see if 5-fluorouracil-resistant bacteria and neoplasms could form 5-fluorouridylic acid. Reichard,

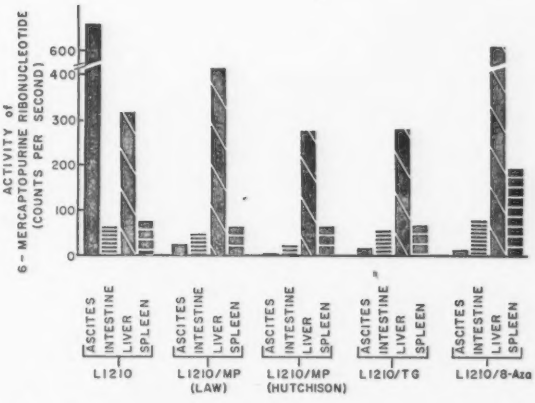



**Fig. 6.** Conversion of 6-mercaptopurine-$S^{35}$ to the ribonucleotide in vivo by ascites tumor cells that are sensitive *(L1210)* or resistant *(L1210/MP)* to inhibition by 6-mercaptopurine.[23] The 6-thioguanine-resistant *(L1210/TG)* and 8-azaguanine-resistant *(L1210/8-Aza)* neoplasms are cross-resistant to 6-mercaptopurine.

*That is, ribonucleic acid and protein synthesis continues unabated whereas deoxyribonucleic acid synthesis, hence cell division, is stopped by lack of thymidylic acid.

Sköld, and Klein[135] observed that 5-fluorouracil-resistant Ehrlich ascites tumor cells had lost the capacity to catalyze the conversion of uracil to uridine—the first step in formation of the nucleotide in mammalian cells. Studies in progress in this laboratory, in collaboration with Dr. L. W. Law of the National Cancer Institute, have shown that 5-fluorouracil-resistant and 5-fluorouridine-resistant P815 mast cell neoplasms have a significantly decreased capacity to form nucleotide derivatives of 5-fluorouracil relative to the sensitive line; however, the resistant cells have not lost this capacity.[22] In studies with bacteria that are profoundly resistant to 5-fluorouracil, it was found in this laboratory that such cells fail to form nucleotide derivatives of uracil and 5-fluorouracil. Orotic acid, however, is still utilized for pyrimidine synthesis by 5-fluorouracil-resistant cells. In certain bacteria, it appears that uracil can be converted directly to uridylic acid by reaction with 5-phosphoribosylpyrophosphate.[42] The strain of *Escherichia coli* used in experiments in this laboratory could accomplish this synthesis to a limited extent. A comparison of the capacity of enzyme preparations from sensitive and resistant bacteria to catalyze formation of uridylic, 5-fluorouridylic, orotidylic, and 5-fluoroorotidylic acids via this pathway revealed that 5-fluorouracil-resistant cells had lost the enzyme capacity to form uridylic and 5-fluorouridylic acids but not the enzyme capacity to form orotidylic and 5-fluoroorotidylic acids.[19] These organisms could still be inhibited with 5-fluoroorotic acid, which is metabolized to 5-fluorouridylic acid derivatives by a different pathway (Fig. 8). Studies using strains of *S. faecalis* resistant to 5-fluorouridine, 5-fluoro-2′-deoxyuridine, and 5-fluoroorotic acid have revealed that these organisms are resistant to 5-fluorouracil and that none of them can convert 5-fluorouracil to nucleotide derivatives.[27]

It is evident that resistance to 5-fluorouracil can arise, as in the case of purine analogues, by loss of capacity of resistant cells to carry out a lethal synthesis. That other mechanisms of resistance to 5-fluorouracil may exist appears evident from the work of Heidelberger and co-workers,[72, 73, 76] who observed that a line of 5-fluorouracil-resistant Ehrlich ascites tumor cells could still synthesize 5-fluoro-2′-deoxyuridylic acid but that this compound no longer inhibited the conversion of deoxyuridylic acid to thymidylic acid by an enzyme preparation from resistant cells. Enzymes from sensitive cells were inhibited by 5-fluoro-2′-deoxyuridylic acid. These results were interpreted to mean that resistance in this case was a consequence of decreased affinity of thymidylate synthetase* for 5-fluoro-2′-deoxyuridylic acid.[76] Results of these studies suggest that multiple mechanisms of resistance to 5-fluorouracil can exist in experimental neoplasms.

### Resistance to analogues of glutamine which can inhibit purine biosynthesis

The work on the biosynthesis of purines in the laboratories of Buchanan and Hartman[30, 69] and Greenberg and Jaenicke[62] is a recognized landmark of biochemistry. Fig. 4*A* outlines this pathway, and Fig. 4*B* shows the interconversions of purine ribonucleotides (see references 69 and 78 for recent reviews of literature on ribonucleotide interconversions).

***Azaserine and DON.*** Azaserine, the first antibiotic known to possess anticancer activity against experimental neoplasms,[59, 150] was shown to inhibit the incorporation of formate-$C^{14}$ into the nucleic acids of mouse neoplasms in vivo,[14, 143] to inhibit purine biosynthesis by pigeon liver extracts,[70] and then, more specifically, to inhibit the formation of formylglycinamidine ribotide,[104, 152] as indicated in Fig. 4*A*. DON (Fig. 1), a structurally related antibiotic, inhibits by this same mechanism and is an even more potent glutamine antagonist than azaserine. Higher levels of azaserine and DON also inhibit other steps in purine biosynthesis in which glutamine

*The enzyme which catalyzes synthesis of thymidylic acid from deoxyuridylic acid.

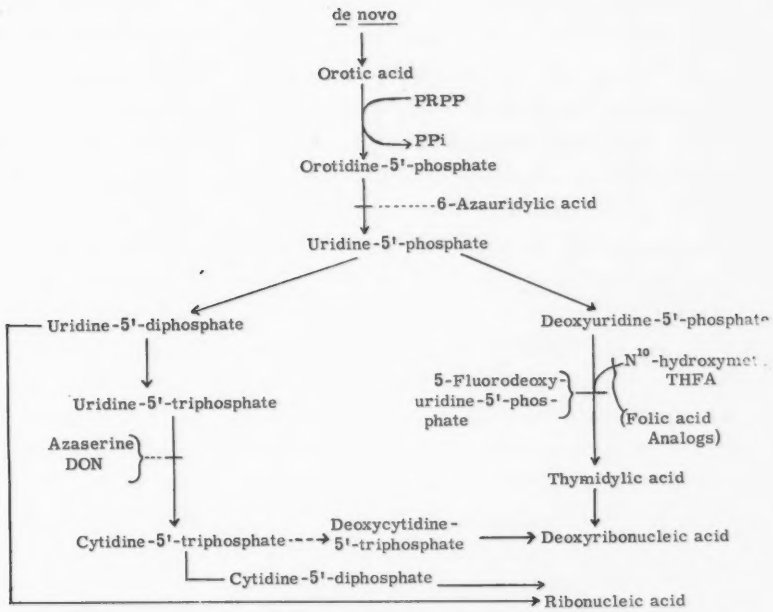



**Fig. 7.** Map of pyrimidine biosynthesis (see reference 134). Fluorouracil has been shown to inhibit uridine synthesis,[135] and 5-fluorodeoxyuridylic acid is a potent inhibitor of thymidylic acid synthesis[41]; folic acid analogues inhibit thymidylic acid synthesis. Glutamine antagonists inhibit amination of uridine-5'-triphosphate in mammalian cells.[1, 52, 90, 91] *THFA*, tetrahydrofolic acid; *PRPP*, 5-phosphoribosyl-1-pyrophosphate; *PPi*, inorganic pyrophosphate.

acts as a nitrogen donor. For example, the synthesis of 5-phosphoribosylamine is inhibited[105]; at even higher concentrations of azaserine, the conversion of xanthylic acid to guanylic acid can also be inhibited,[1] as shown in Figs. 4*A* and 4*B*. In some biologic systems, it can be shown that azaserine and DON inhibit the synthesis of cytidine nucleotides from uracil nucleotides[1, 52, 90, 91] (see Fig. 7). All of these reactions that are inhibited involve glutamine and are on pathways leading to synthesis of purine and pyrimidine nucleotides and hence to nucleic acids.

LePage and co-workers[63, 121] showed that azaserine was bound with a component of ascites tumor cells, presumably an enzyme. Recent work by Herrmann, Day, and Buchanan[79] has shown conclusively that azaserine reacts witih a purified preparation of the enzyme which catalyzes the synthesis of formylglycinamidine ribonucleotide. Studies of resistance to azaserine in TA3 carcinoma suggested that the resistant cell

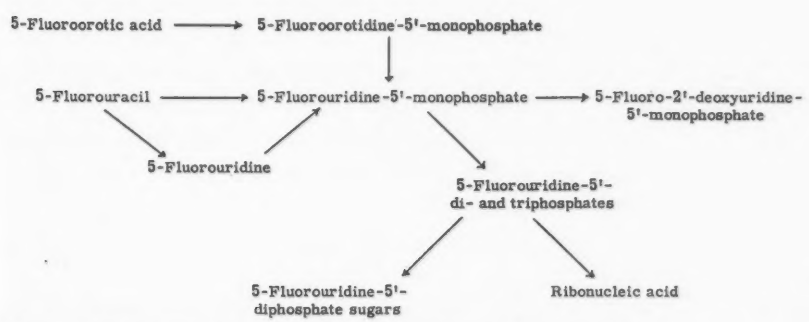



**Fig. 8.** Anabolism of 5-fluoropyrimidines (see reference 68).

line recovered more rapidly from the inhibitory effect of the drug and also indicated an increased capacity of resistant cells to utilize preformed purines for nucleic acid synthesis.[139] Resistance to azaserine and DON in experimental neoplasms was observed to develop stepwise and, in some instances, in a single step.[100, 131, 132]

Anderson, Levenberg, and Law[6] showed that purine bioysnthesis was inhibited by azaserine in intact cells of the sensitive line of mouse plasma cell neoplasm 70429, whereas such inhibition was less in cells of an azaserine-resistant subline. It was found that the enzyme systems present in cell-free extracts from sensitive and from resistant 70429 catalyzed purine biosynthesis equally well. Azaserine and DON inhibited purine biosynthesis in cell-free extracts from both sensitive and resistant 70429. It thus appears that resistance to these inhibitors is at least partly a function of the intact cell, and one might be tempted to propose decreased cell permeability as a resistance mechanism. This interpretation has not been placed on these results.[5, 6] It is evident from the work of Pine[130] that azaserine is taken up by both sensitive and resistant 70429; Jacquez and Hutchison[86] have observed uptake of azaserine and DON by both sensitive and resistant lines of other neoplasms. It is not possible as yet to state a precise mechanism of resistance to azaserine and DON. Resistance to these agents may be a consequence of binding within the resistant cell in such a way that the inhibitors do not reach the sensitive enzyme system; a similar mechanism of resistance to amethopterin has been postulated.[8]

### Resistance to folic acid analogues

The folic acid analogue aminopterin is the first metabolite antagonist found to be clinically useful in the treatment of neoplastic disease.[56] Amethopterin* is the folic acid analogue now most generally used in the treatment of acute leukemia (Fig. 1).

*Methotrexate.

Studies of the role of folic acid in metabolism revealed that it functioned in utilization of a one carbon unit (here conveniently referred to as formate) in synthesis of amino acids and of labile methyl groups. Skipper, Mitchell, and Bennett[144] demonstrated that folic acid analogues inhibited the incorporation of formate into nucleic acid purines, and Totter[154] showed that amethopterin inhibited the synthesis of deoxyribonucleic acid thymine. These studies have been extended in recent years until it is now possible to point to the specific sites of inhibition by folic acid analogues of the synthesis of inosinic acid (Fig. 4A) and of thymidylic acid (Fig. 7), as well as to other consequences of inhibition of the formation of cofactor forms of folic acid.[81] The earlier observation on the inhibition by folic acid analogues of the conversion of folic acid to cofactor forms* active in formyl group transfer has more recently been extended to studies with isolated enzymes by Osborn, Freeman, and Huennekens[127a] and by Zakrewski and Nichol.[162] The formation of active forms of folic acid was not inhibited by folic acid analogues in bacterial cells resistant to the inhibitors.[8, 125, 157]

As mentioned earlier, amethopterin-resistant mouse neoplasms have been selected by exposure to the drug; resistance to amethopterin has been demonstrated to develop by successive stepwise mutations leading to genetically stable resistant neoplasms.[96, 99] This process appears to be analogous to the stepwise development of resistance to penicillin in bacteria and has recently been observed in isolation of amethopterin-resistant lines of mammalian cells in tissue culture.[9] *S. faecalis* mutants resistant to concentrations of amethopterin many times greater than drug levels required to inhibit the parent drug-sensitive strain have been isolated and studied in detail.[8, 83, 85, 124, 125, 127] One mechanism of resistance postulated was that of decreased

*Huennekens and Osborn[81] have recently presented an excellent review of this complex subject.

cell permeability.[124, 127] However, Wacker, Ebert, and Kolm[157] demonstrated that as much and more aminopterin penetrated resistant bacterial cells as infiltrated sensitive cells. The earlier brief reports of Johnson and associates[88] and Anton and Nichol[7] are in agreement with Wacker's observation.

A recent report by Anton and Nichol[8] poses an interesting problem analogous to that encountered in resistance to azaserine. It appears that the enzyme systems extracted from resistant bacteria which catalyze the conversion of folic acid to active cofactors for formyl group transfer are almost as sensitive to inhibition by amethopterin as are similar enzyme preparations from drug-sensitive bacteria. In contrast to results of earlier studies,[124, 127] these amethopterin-resistant cells incorporated more of the inhibitor than did the sensitive cells. The interpretation placed on these data is that the amethopterin which gains entry into resistant cells is bound in an inactive form or is in some other way prevented from reaching the sensitive enzyme system. It remains to be demonstrated whether such a mechanism holds in resistant neoplasms.*

### Other mechanisms of resistance

It would be misleading to imply that the mechanisms of resistance to drugs mentioned so far are the only ones known. The following listing is based on the carefully considered mechanisms of resistance to drugs presented by Davis and Maas[47, 109] and by Nichol.[125]

1. Cell permeability—decreased penetration of the drug into the cell.
2. Detoxification—increased destruction or inactivation of the drug or binding of the drug within the cell in an inactive form.
3. Decreased activation—decreased conversion of an administered compound into a more active inhibitor.
4. Increased metabolite or enzyme—increased concentration of a metabolite antagonizing the drug or an increased formation of the enzyme inhibited by the drug.
5. Decreased need for end product—decreased quantitative requirement for a product of the metabolite.
6. Alternate metabolic pathway—essential metabolite produced by a different pathway that bypasses the site of inhibitory action of the drug.
7. Altered enzyme affinity—decreased affinity of the sensitive enzyme for the drug relative to the enzyme affinity for the metabolite.

The metabolism of an analogue to yield an inhibitory derivative (lethal synthesis) and the possibility of resistance coming about by a diminution or deletion of enzyme(s) for this metabolism would fall in the third category listed above, and clear illustrations of this mechanism have been presented during the course of this discussion. Although the emphasis herein on resistance to purine analogues has centered around this particular resistance mechanism, one should not lose sight of the probability that mutation-selection provides the basis for the elaboration of multiple mechanisms of resistance to a given drug. Drug resistance in neoplasms is known to arise in stepwise fashion, probably as the result of discrete mutational events. In most experimental work, the extreme cases are studied, that is, the sensitive and the highly resistant cell lines are compared. Study of intermediate levels of resistance to drugs may reveal still other mechanisms.

*Since this manuscript was prepared, results of studies on the mechanism of resistance to amethopterin in mammalian cells have been presented. Dr. G. A. Fischer observed an increase in the folic acid reductase level of amethopterin-resistant L5178 mouse leukemia cells in culture (Proc. Am. A. Cancer Res. 3:111, 1960). The increased level of folic acid reductase paralleled the increased amounts of amethopterin required for inhibition. Drs. M. T. Hakala, S. F., Zakrzewski, and C. A. Nichol observed that the capacity of sarcoma 180 cells in culture to bind amethopterin was greatly increased in the resistant lines; this increased binding of the inhibitor was related to an increase in the amount of folic acid reductase in the cells (Proc. Am. A. Cancer Res. 3:115, 1960). It thus appears that amethopterin may be irreversibly bound to folic acid reductase and that cells that produce large amounts of this enzyme become resistant by a binding of the inhibitor by the excess enzyme. If the increased production of folic acid reductase results from enzyme induction, it might be possible to discover ways of inhibiting such induction and thus eliminate the development of resistance by this mechanism.

### Cross-resistance and collateral sensitivity

It has been observed, particularly in the area of purine analogues, that development of resistance to one purine analogue, 6-mercaptopurine for example, results in simultaneous development of resistance to other purine analogues—6-thioguanine and 8-azaguanine.[100, 102] In the earlier discussion of resistance in bacterial cells to purine analogues, it was pointed out that capacity to convert hypoxanthine, 6-mercaptopurine, guanine, and 8-azaguanine to ribonucleotides seemed to be lost simultaneously in resistant bacterial cells but that such cells were still capable of forming adenylic acid; these results presumably depend upon the specificity of the enzymes involved. It is significant that studies of metabolism of these same purines in the L1210 leukemia lines of Law that are resistant to purine analogues gave the same result.[17, 23] This result provides a basis for understanding the observed cross-resistance between 6-mercaptopurine, 6-thioguanine, and 8-azaguanine[100] and may explain the observation that an adenine isomer, 4-aminopyrazolo (3,4-d) pyrimidine, can inhibit neoplasms resistant to other purine analogues.[146]

Azaserine and DON are structural analogues, and both are glutamine antagonists (Fig. 1). It is therefore not surprising that resistance to one drug results in resistance to the other. Neoplasms resistant to purine analogues retain sensitivity to glutamine antagonists and vice versa. This can readily be understood, since these two classes of inhibitors act at quite different sites (Figs. 4*A* and 4*B*).

An example of collateral sensitivity* is the apparent increased sensitivity of mouse neoplasms that are resistant to purine analogues to inhibition by amethopterin.[100, 102] It is interesting that the reverse situation does not hold and that amethopterin-resistant neoplasms do not appear to be any more sensitive to purine analogues than is the parent line and may be even less so.[148*]

The pyrimidine analogues inhibit by entirely different mechanisms from the other inhibitors discussed above in this section, and hence 5-fluorouracil or its nucleoside derivatives would be expected to inhibit those neoplasms resistant to the other anticancer agents under discussion—provided, of course, that the parent line of the neoplasm responded to fluorouracil. This appears to be the case from experimental observations. For example, 6-mercaptopurine-resistant L1210 leukemia, amethopterin-resistant L1210, and azaserine-resistant and DON-resistant P815 mast cell neoplasms are all sensitive to 5-fluorouracil.†

### Circumvention of resistance to drugs

At the outset it must be said that this goal has not yet been achieved. Increasing knowledge of mechanisms of action of temporarily effective anticancer agents and an understanding of the biochemical basis of resistance to anticancer agents may suggest means of circumventing drug resistance. For example, from knowledge that 6-mercaptopurine is converted to a nucleotide and from knowledge that this product of its metabolism is inhibitory comes the suggestion that 6-mercaptopurine ribonucleotide might inhibit the 6-mercaptopurine-resistant neoplasm. This compound, 6-mercaptopurine ribonucleotide, has now been synthesized, but unfortunately most cells appear to be impermeable to nucleotides. For example, certain bacteria capable of utilizing purine bases or purine ribonucleosis for growth are unable to grow on purine ribonucleotides. Mammalian cells appear to break nucleotides down to the nucleoside or free base level before utilizing them. The skill of the organic chemist may yet devise a means of masking the ionized nucleotide and introducing this Trojan horse into the 6-mercaptopurine-resistant cell. Still other tech-

*Collateral sensitivity can be defined as *increased* sensitivity to an inhibitor or class of inhibitors as a consequence of the development of resistance to a different class of inhibitors.

*L. W. Law: Personal communication.

**Table II.** *Summary of proposed mechanisms of resistance to certain antimetabolites*

| *Inhibitor* | *Active form* | *General area of inhibition* | *Recognized site of inhibition* | *Postulated or recognized mechanism of resistance* |
|---|---|---|---|---|
| Azaserine | Azaserine | Purine biosynthesis | Formylglycinamidine ribonucleotide synthesis | Failure of inhibitor to reach the sensitive enzyme (postulated)* |
| Amethopterin | Amethopterin | Purine, thymine, and serine biosynthesis | Synthesis of cofactor forms of folic acid | |
| 6-Mercaptopurine | Nucleotide | Purine ribonucleotide interconversions; purine biosynthesis | Conversions of inosinic acid to adenylic and guanylic acids; de novo purine biosynthesis | |
| 6-Thioguanine† | Nucleotide | Purine ribonucleotide interconversions; purine biosynthesis | Similar to 6-mercaptopurine | Resistant cells fail to form inhibitory amounts of the nucleotide |
| 8-Azaguanine† | Nucleotide | Protein biosynthesis | Incorporation of cystine and methionine into protein | |
| 6-Azauracil† | Nucleotide | Pyrimidine biosynthesis | Formation of uridylic acid | |
| 5-Fluorouracil†‖ | Deoxyribonucleotide | Thymine biosynthesis | Conversion of deoxyuridylic acid to thymidylic acid | 1. Failure to form nucleotide derivative‡<br>2. Decreased affinity of sensitive enzyme for inhibitor§ |

*This may be a result of binding of inhibitor in an inactive form within the cell.[8] Altered enzyme affinity may also be a factor in resistance to these agents in some biologic systems.

†These agents are known to be incorporated into ribonucleic acid in place of the corresponding metabolite and may thereby exert indirect inhibitory effects. This may be particularly true in the case of 8-azaguanine.

‡This mechanism was observed in 5-fluorouracil-resistant Ehrlich ascites tumor cells[135] and in resistant bacteria.[19]

§This mechanism was observed in 5-fluorouracil-resistant Ehrlich ascites tumor cells.[76]

‖It has recently been observed by F. Gros (see Chantrenne, H.: Nature 184: 1198, 1960) that 5-fluorouracil inhibits protein synthesis in *E. coli* and that this inhibitor specifically reduces fixation of proline and of tyrosine on soluble ribonucleic acid.

niques may be devised for introducing such inhibitors into cells. For example, Anand and coauthors[3, 3a] have recently described an effect of streptomycin on cell membranes and observed that nucleotides leak out of streptomycin-damaged bacteria. It might be possible to introduce nucleotides into cells by altering membrane permeability in some way; thought might profitably be given to devices for introducing 6-mercaptopurine ribonucleotide into 6-mercaptopurine-resistant leukemic cells.

Restoration of enzyme activity in a resistant cell would seem to be possible if this loss of enzyme activity were due to lack of a cofactor or to the presence of some inhibitor of enzyme action which could be removed. If, as seems likely in some instances, loss of enzyme activity is a consequence of gene mutation followed by en-

zyme deletion, restoration of enzyme activity would require addition of the enzyme itself. This would appear to be an unlikely prospect. Even so, one can indulge in speculation as to the possibility of lysing a limited number of red blood cells, thus releasing enzymes from these cells some of which are known to be capable of catalyzing formation of 6-mercaptopurine ribonucleotide. It might appear unlikely that an intact enzyme, being a high molecular weight protein molecule, could gain entry into leukemic cells, and yet high molecular weight substances such as deoxyribonucleic acid ("transforming principle") are known to pass bacterial cell membranes and polypeptides of moderately high molecular weight are known to gain entry into mammalian cells.

At the present time, the most feasible approach to circumvention of resistance appears to be through use of combinations of inhibitors which act at different sites*—a subject which was considered in detail recently by LePage.[103a] Potter[133] has discussed the possibilities for "sequential blocking"—use of combinations of compounds which inhibit reactions on the same pathway. For example, azaserine in combination with 6-mercaptopurine appeared to be more effective in prolonging the life span of leukemic mice than higher levels of either drug alone,[33, 39] and combinations of amethopterin with purine analogues gave pronounced increases in life span of leukemic mice.[97, 149] By reference to Fig. 4, it can be seen that these agents act in sequence on the same biochemical pathway. Burchenal[32] has reported clinical results which suggest that combination therapy may delay the appearance of resistance to 6-mercaptopurine. The real need is for combinations of inhibitors with selective toxicity for cancer cells in order that the clinician may have a wider therapeutic index within which to work. Knowledge of mechanisms of drug inhibition and drug resistance provide a rational basis for approaching the selection of potentially useful combinations of agents. From a theoretic standpoint, the probability of a cell becoming resistant by mutation-selection to two drugs administered simultaneously is much less than the probability of development of resistance to a single drug.

In a series of interesting experiments employing mouse leukemia as a research tool, Goldin and colleagues[61] and Skipper and associates[147] were able to show that the number of leukemic cells inoculated into mice was a definite factor in "curability." That is, if a large number of L1210 leukemic cells ($1 \times 10^6$ cells) were inoculated, one could increase the life span of amethopterin-treated animals by 100 per cent of untreated controls, but all animals eventually died of leukemia. By dropping the number of cells inoculated to $1 \times 10^3$, it was observed that 60 per cent of the amethopterin-treated animals were alive and healthy 60 days later. This percentage of "cures" rose to 80 per cent when $1 \times 10^2$ cells were inoculated. If the mutation rate in such a cell population is of the order of 1 cell in $10^5$, the probability of a given mouse receiving a drug-resistant cell arising by a mutational event would be a function of the size of initial inoculum. In clinical cases, the leukemic cell count may well be extremely high before any therapy is begun. One implication of these studies—keeping in mind that data from idealized mouse leukemia experiments were cautiously extrapolated—is that "an effort might be made to depress the total number of leukemic cells in the host to the lowest practical number with one agent and immediately follow such therapy with a second agent that acts at a different biochemical site."[147] The possibility exists that such a protocol might be more successful than that customarily used, namely, intermittent therapy with a single drug until a refractory state develops. It should also be mentioned that the use of chemotherapeutic agents in combination with surgical

*Woodruff and McDaniel[161] presented an interesting discussion of synergism and potentiation of combinations of antibiotics. The principles involved are also applicable to combination therapy with other inhibitors.

procedures and radiation may prove of value in the therapy of cancer and that such techniques are being evaluated.

Welch[160] has recently discussed the problem of "sequestering" of leukemic cells in sites inaccessible to drugs—for example, in the spinal column and brain—and the value of intrathecal chemotherapy in addition to intravenous therapy in the treatment of leukemia.

### Conclusions

A biochemical basis for resistance to certain purine and pyrimidine analogues which possess anticancer activity is known. In the case of the clinically useful agents, such as 6-mercaptopurine and 5-fluorouracil, one mechanism of resistance in experimental neoplasms in mice is an enzymic failure of resistant cells to convert these analogues to nucleotide derivatives—an event viewed as a lethal synthesis. Other mechanisms of resistance are known, and it has been concluded that resistance to 5-fluorouracil can also arise as a consequence of a decreased sensitivity or affinity of an enzyme system for an active form of the inhibitor, 5-fluoro-2′-deoxyuridylic acid.[76] Precise mechanisms of resistance to amethopterin and azaserine in experimental neoplasms are now being delineated. The mechanisms of inhibition and resistance to inhibitors of nucleic acid synthesis that were considered in some detail in this presentation are summarized in Table II. It remains to be demonstrated whether or not these resistance mechanisms occur in human cancer. Such studies appear to be feasible in the case of leukemia.*

*In this laboratory, in collaboration with Dr. R. R. Ellison and Dr. J. H. Burchenal, Sloan-Kettering Institute, and Dr. S. D. Palmer, University Hospital, University of Alabama, a study of the capacity of enzyme preparations from human leukemic cells to catalyze formation of ribonucleotides of natural purines and 6-mercaptopurine has shown that such enzyme preparations do catalyze these reactions (Fig. 3). Dr. J. D. Davidson, National Cancer Institute, in independently conceived studies, has made similar observations. It should be pointed out that red blood cells also actively catalyze nucleotide formation and one of the problems encountered in such a study is the selective removal of essentially all red cells without damaging the leukemic cells.

Increasing knowledge of mechanisms of inhibition of anticancer agents and of the biochemical mechanisms of resistance to such agents provides a basis for devising means to circumvent drug resistance. Such knowledge is considered essential for a rational approach to cancer chemotherapy.

The author is indebted to associates at Southern Research Institute for able technical assistance and to Dr. H. E. Skipper and Dr. L. L. Bennett, Jr., for their continued interest in work on drug resistance. Discussions and collaborative research with Dr. Dorris J. Hutchison, Sloan-Kettering Institute, on microbiologic aspects and with Dr. Elizabeth P. Anderson and Dr. Lloyd W. Low, National Cancer Institute, on, respectively, enzymologic and biologic aspects of drug resistance have been invaluable.

### *References*

1. Abrams, R., and Bentley, M.: Biosynthesis of nucleic acid purines. III. Guanosine 5′-phosphate formation from xanthosine 5′-phosphate and L-Glutamine, Arch. Biochem. & Biophys. **79**:91-110, 1958.
2. Albert, A.: Selective toxicity, New York, 1951, John Wiley & Sons, Inc.
3. Anand, N., and Davis, B. D.: Damage by streptomycin to the cell membrane of *Escherichia coli*, Nature, London **185**:22-23, 1960.

3a. Anand, N., Davis, B. D., and Armitage, A. K.: Uptake of streptomycin by *E. coli*, Nature, London **185**:23-24, 1960.

4. Anderson, E. P., Kalckar, H. M., and Isselbacher, K. J.: Defect in uptake of galactose-1-phosphate into liver nucleotides in congenital galactosemia, Science **125**:113-114, 1957.
5. Anderson, E. P., and Law, L. W.: Biochemistry of cancer, Ann. Rev. Biochem. **29**:577-608, 1960.
6. Anderson, E. P., Levenberg, B., and Law, L. W.: Purine biosynthesis in azaserine-sensitive and -resistant lines of the mouse plasmal cell neoplasm 70429, Fed. Proc. **16**:145, 1957.
7. Anton, A. H., and Nichol, C. A.: Some characteristics of amethopterin-resistant strains of *S. faecalis*, Proc. Am. A. Cancer Res. **2**:91, 1956.
8. Anton, A. H., and Nichol, C. A.: Studies on the nature of resistance of *Streptococcus faecalis* to folic acid antagonists, Biochem. Pharmacol. **3**:1-9, 1959.
9. Aronow, L.: Studies on drug resistance in mammalian cells. I. Amethopterine resistance in mouse fibroblasts, J. Pharmacol. & Exper. Therap. **127**:116-121, 1959.

10. Balis, M. E., Hylin, V., Coultas, M. K., and Hutchison, D. J.: Metabolism of resistant mutants of *Streptococcus faecalis*. II. Incorporation of exogenous purines, Cancer Res. **18**: 220-225, 1958.
11. Balis, M. E., Hylin, V., Coultas, M. K., and Hutchison, D. J.: Metabolism of resistant mutants of *Streptococcus faecalis*. III. The action of 6-mercaptopurine, Cancer Res. **18**: 440-444, 1958.
12. Balis, M. E., Levin, D. H., Brown, G. B., Elion, G. B., Nathan, H. C., and Hitchings, G. H.: The effects of 6-mercaptopurine on *Lactobacillus casei*, Arch. Biochem. & Biophys. **71**:358-366, 1957.
13. Beadle, G. W.: Biochemical genetics, Chem. Rev. **37**:15-96, 1945.
14. Bennett, L. L., Jr., Schabel, F. M., Jr., and Skipper, H. E.: Studies on the mode of action of azaserine, Arch. Biochem. & Biophys. **64**:423-436, 1956.
15. Bennett, L. L., Jr., Skipper, H. E., and Law, L. W.: Incorporation of 8-azaguanine-2-$C^{14}$ into 8-azaguanine-susceptible and 8-azaguanine-dependent leukemic tumors, Fed. Proc. **12**:300, 1953.
16. Bosch, L., Harbers, E., and Heidelberger, C.: Studies on fluorinated pyrimidines. V. Effects on nucleic acid metabolism in vitro, Cancer Res. **18**:335-343, 1958.
17. Brockman, R. W., Bennett, L. L., Jr., Simpson, M. S., Wilson, A. R., Thomson, J. R., and Skipper, H. E.: A mechanism of resistance to 8-azaguanine. II. Studies with experimental neoplasms, Cancer Res. **19**:856-869, 1959.
18. Brockman, R. W., Bennett, L. L., Jr., and Skipper, H. E.: A comparison of purine metabolism in strains of *Streptococcus faecalis* susceptible and resistant to 6-mercaptopurine inhibition, Proc. Am. A. Cancer Res. **2**:191-192, 1957.
19. Brockman, R. W., Davis, J. M., and Stutts, P.: Metabolism of uracil and 5-fluorouracil by drug-sensitive and by drug-resistant bacteria, Biochim. et biophys. acta **40**:22-32, 1960.
20. Brockman, R. W., and Hutchison, D. J.: Decreased ribonucleotide pyrophosphorylase activity in *S. faecalis* mutants resistant to purine analogs, Fed. Proc. **18**:197, 1959.
21. Brockman, R. W., Hutchison, D. J., and Skipper, H. E.: Metabolism of purines and purine antagonists by drug-resistant strains of *Streptococcus faecalis*, Fed. Proc. **17**:195, 1958.
22. Brockman, R. W., and Law, L. W.: Metabolism of pyrimidines and 5-fluoropyrimidines by sensitive and resistant neoplasms, Proc. Am. A. Cancer Res. **3**:98, 1960.
23. Brockman, R. W., Simpson, M. S., Davis, J. M., and Stutts, P.: A mechanism of resistance to 6-mercaptopurine: Metabolism of hypoxanthine and 6-mercaptopurine by sensitive and resistant neoplasms, Cancer Res. **20**:643-653, 1960.
24. Brockman, R. W., Sparks, C. S., Stutts, P., and Schabel, F. M., Jr.: Biochemical basis for resistance to purine antagonists in *Streptococcus faecalis*, Bacteriol. Proc., p. 52, 1959.
25. Brockman, R. W., Sparks, C. S., Hutchison, D. J., and Skipper, H. E.: A mechanism of resistance to 8-azaguanine. I. Microbiological studies on the metabolism of purines and 8-azapurines, Cancer Res. **19**:177-188, 1959.
26. Brockman, R. W., Sparks, M. C., and Simpson, M. S.: A comparison of the metabolism of purines and purine analogs by susceptible and drug-resistant bacterial and neoplastic cells, Biochim. et biophys. acta **26**:671-672, 1957.
27. Brockman, R. W., and Sparks, C. S.: Metabolism of pyrimidines and 5-fluoropyrimidines by drug-sensitive and drug-resistant *Streptococcus faecalis*, Bacteriol. Proc., p. 71, 1960.
28. Brockman, R. W., Sparks, C. S., Simpson, M. S., and Skipper, H. E.: Decreased ribonucleotide pyrophosphorylase activity of *Streptococcus faecalis* and L1210 leukaemia resistant to purine antagonists, Biochem. Pharmacol. **2**:77-79, 1959.
29. Brockman, R. W., and Stutts, P.: A mechanism of resistance to 6-thioguanine, Fed. Proc., 1960.
30. Buchanan, J. M., and Hartman, S. C.: Enzymic reactions in synthesis of purines, Advances Enzymol. **21**:199-261, 1959.
31. Burchenal, J. H.: Cell lines developing resistance, *in* Wolstenholme, G. E. W., and O'Connor, C. M., editors: Symposium on chemotherapy of cancer, Proceedings of the Third National Cancer Congress, Philadelphia, 1956, J. B. Lippincott Company, pp. 413-420.
32. Burchenal, J. H.: Current status of clinical chemotherapy, Current Res. Cancer Chemother. **4**:3-30, 1956.
33. Burchenal, J. H., Murphy, M. C., Yuceoglu, M., and Horsfall, M.: The effect of a new antimetabolite, O-diazoacetyl-L-serine, in mouse leukemia, Proc. Am. A. Cancer Res. **1**:7, 1954.
34. Burchenal, J. H., Robinson, E., Johnston, S. F., and Kushida, M. M.: The induction of resistance to 4-amino-$N^{10}$-methylpteroylglutamic acid in a strain of transmitted mouse leukemia, Science **111**:16, 1950.
35. Chaudhuri, N. K., Montag, B. J., and Heidel-

berger, C.: Studies on fluorinated pyrimidines. III. The metabolism of 5-fluorouracil-2-$C^{14}$ and 5-fluoroorotic-2-$C^{14}$ acid in vivo, Cancer Res. **18**:318-328, 1958.

36. Chaudhuri, N. K., Mukherjee, K. L., and Heidelberger, C.: Studies on fluorinated pyrimidines. VII. The degradative pathway, Biochem. Pharmacol. **1**:328-341, 1958.
37. Clarke, D. A., Elion, G. B., Hitchings, G. H., and Stock, C. C.: Structure-activity relationships among purines related to 6-mercaptopurine, Cancer Res. **18**:445-456, 1958.
38. Clarke, D. A., Philips, F. S., Sternberg, S. S., Hitchings, G. H., Stock, C. C., and Elion, G. B.: 6-Mercaptopurine: Effects in mouse sarcoma 180 and in normal animals, Cancer Res. **13**:593-604, 1953.
39. Clarke, D. A., Philips, F. S., Stock, C. C., Elion, G. B., and Hitchings, G. H.: Combined chemotherapy of mouse sarcoma 180 (S180), azaserine, and 6-mercaptopurine, *in* Abstracts of the 125th meeting, American Chemical Society, Kansas City, 1954, p. 12M.
40. Cohen, S. S.: Unbalanced growth and death: A study in thymine metabolism, *in* Graff, S., editor: Essays in biochemistry, New York, 1956, John Wiley & Sons, Inc., pp. 77-84.
41. Cohen, S. S., Flaks, J. G., Barner, H. D., Loeb, M. R., and Lichtenstein, J.: The mode of action of 5-fluorouracil and its derivatives, Proc. Nat. Acad. Sc. **44**:1004-1012, 1958.
42. Crawford, I., Kornberg, A., and Simms, E. S.: Conversion of uracil and orotate to uridine 5′-phosphate by enzymes in Lactobacilli, J. Biol. Chem. **226**:1093-1101, 1957.
43. Danneberg, P., Montag, B. J., and Heidelberger, C.: Studies on fluorinated pyrimidines. IV. Effects on nucleic acid metabolism in vivo, Cancer Res. **18**:329-334, 1958.
44. Davidson, J. D.: Permeability of resistant L1210 leukemia cells to 8-azaguanine and 6-mercaptopurine, Proc. Am. A. Cancer Res. **2**:290-291, 1958.
45. Davidson, J. D.: Mechanism of action of 6-mercaptopurine in L1210 leukemia, Proc. Am. A. Cancer Res. **3**:15, 1959.
46. Davidson, J. D.: Studies on the mechanism of action of 6-mercaptopurine in sensitive and resistant L1210 leukemia in vitro, Cancer Res. **20**:225-232, 1960.
47. Davis, B. D.: Physiological (phenotypic) mechanisms responsible for drug resistance, *in* Wolstenholme, G. E. W., and O'Connor, C. M., editors: Ciba Foundation symposium on drug resistance in microorganisms, Boston, 1957, Little, Brown & Company, pp. 165-179.
48. Domagk, G.: Chemotherapy of bacterial infections, Deutsche med. Wchnschr. **61**:250-253, 1935.
49. Ehrlich, P.: Über den jetzigen Stand der Chemotherapie, Ber. deutsch. chem. Gesellsch. **42**: 17-47, 1909.
50. Ehrlich, P., and Hata, S.: Die experimentelle Chemotherapie der Spirilosen, Berlin, 1911.
51. Ehrlich, P.: Address in pathology on: Chemotherapeutics, principles, methods, and results, Lancet **2**:445-451, 1913.
52. Eidinoff, M. L., Knoll, J. E., Morano, B., and Cheong, L.: Pyrimidine studies. I. Effect of DON (6-diazo-5-oxo-L-norleucine) on incorporation of precursors into nucleic acid pyrimidines, Cancer Res. **18**:105-109, 1958.
53. Elion, G. B., Burgi, E., and Hitchings, G. H.: Studies on condensed pyrimidine systems. IX. The synthesis of some 6-substituted purines, J. Am. Chem. Soc. **74**:411-414, 1952.
54. Elion, G. B., Singer, S., and Hitchings, G. H.: The purine metabolism of a 6-mercaptopurine-resistant *Lactobacillus casei*, J. Biol. Chem. **204**:35-41, 1953.
55. Elion, G. B., Singer, S., and Hitchings, G. H.: An 8-azaguanine-resistant strain of *Lactobacillus casei*, Fed. Proc. **15**:248, 1956.
56. Farber, S., Diamond, L. K., Mercer, R. D., Sylvester, R. F., Jr., and Wolff, J. A.: Temporary remissions in acute leukemia in children produced by folic acid antagonists, 4-aminopteroylglutamic acid (aminopterin), New England J. Med. **238**:787-793, 1948.
57. Fincham, J. R. S.: The biochemistry of genetic factors, Ann. Rev. Biochem. **28**:343-364, 1959.
58. Fizz-Looney, F., and Linderstrøm-Lang, K.: The thermodynamic activity of the male housefly, New York, 1956, Academic Press, Inc.
59. Fusari, S. A., Frohardt, R. P., Ryder, A., Haskell, T. H., Johannessen, D. W., Elder, C. C., and Bartz, Q. R.: Azaserine, a new tumor-inhibitory substance: Isolation and characterization, J. Am. Chem. Soc. **76**:2878-2881, 1954.
60. Garrod, A. E.: Inborn errors of metabolism, London, 1908, Henry Frowde.
61. Goldin, A., Venditti, J. M., Humphreys, S. R., and Mantel, N.: Influence of the concentration of leukemic inoculum on the effectiveness of treatment, Science **123**: 840, 1956.
62. Greenberg, G. R., and Jaenicke, L.: On the activation of the one-carbon unit for the biosynthesis of purine nucleotides, *in* Wolstenholme, G. E. W., and O'Connor, C. M.: editors: Ciba Foundation symposium on chemistry and biology of purines, London, 1957, J. & A. Churchill, Ltd., pp. 204-302.
63. Greenlees, J., and LePage, G. A.: Purine biosynthesis and inhibitors in ascites tumor cells, Cancer Res. **16**:808-813, 1956.
64. Habermann, V., and Šorm, F.: Mechanism of the cancerostatic action of 6-azauracil and its

riboside, Coll. Czech. Chem. Comm. **23**: 2201-2206, 1958.
65. Hampton, A., Maguire, M. H., and Griffiths, J. M.: *in* Abstracts of the International Congress on Biochemistry, 4th meeting, Vienna, 1958, p. 40.
66. Handschumacher, R. E.: Studies of bacterial resistance to 6-azauracil and its riboside, Biochim. et biophys. acta **23**:428-430, 1957.
67. Handschumacher, R. E., and Welch, A. D.: Agents which influence nucleic acid metabolism, *in* Chargaff, E., and Davidson, J. N., editors: The nucleic acids, New York, 1960, Academic Press, Inc., vol. III.
68. Harbers, E., Chaudhuri, N. K., and Heidelberger, C.: Studies on fluorinated pyrimidines. VIII. Further biochemical and metabolic studies, J. Biol. Chem. **234**:1255-1262, 1959.
69. Hartman, S. C., and Buchanan, J. M.: Nucleic acids, purines, pyrimidines (nucleotide synthesis), Ann. Rev. Biochem. **28**:365-410, 1959.
70. Hartman, S. C., Levenberg, B., and Buchanan, J. M.: Involvement of ATP, 5-phosphoribosylpyrophosphate and L-azaserine in the enzymatic formation of glycinamide ribotide intermediates in inosinic acid biosynthesis, J. Am. Chem. Soc. **77**:501-503, 1955.
71. Heidelberger, C., Chaudhuri, N. K., Danneberg, P., Mooren, D., Griesbach, L., Duschinsky, R., Schnitzer, R. J., Pleven, E., and Scheiner, J.: Fluorinated pyrimidines, a new class of tumor-inhibitory compounds, Nature, London **179**:663-666, 1957.
72. Heidelberger, C., Danneberg, P. B., Kaldor, G., and Ghobar, A.: The mechanism of resistance of Ehrlich ascites tumors to 5-fluorouracil, Fed. Proc. **18**:244, 1959.
73. Heidelberger, C., Ghobar, A., Baker, R. K., and Mukherjee, K. L.: Studies on fluorinated pyrimidines. X. In vivo studies on tumor resistance, Cancer Res. **20**:897, 1960.
74. Heidelberger, C., Griesbach, L., Cruz, O., Schnitzer, R. J., and Grunberg, E.: Fluorinated pyrimidines. VI. Effects of 5-fluorouridine and 5-fluoro-2′-deoxyuridine on transplanted tumors, Proc. Soc. Exper. Biol. & Med. **97**:470-475, 1958.
75. Heidelberger, C., Griesbach, L., Montag, B. J., Mooren, D., Cruz, O., Schnitzer, R. J., and Grunberg, E.: Studies on fluorinated pyrimidines. II. Effects on transplanted tumors, Cancer Res. **18**: 305-317, 1958.
76. Heidelberger, C., Kaldor, G., Mukherjee, K. L., and Danneberg, P. B.: Studies on fluorinated pyrimidines. XI. In vitro studies on tumor resistance, Cancer Res. **20**:903, 1960.
77. Heidelberger, C., and Keller, R. A.: The effects of twenty-nine compounds on nucleic acid and protein biosynthesis in slices of Flexner-Jobling carcinoma and rat spleen, *in* Gellhorn, A., and Hirschberg, E., editors: Investigation of diverse systems for cancer chemotherapy screening, Cancer Res. **15**: suppl. 3:106-112, 1955.
78. Heppel, L. A., and Rabinowitz, J. C.: Enzymology of nucleic acids, purines, and pyrimidines, Ann. Rev. Biochem. **27**:613-642, 1958.
79. Herrmann, R. L., Day, R. A., and Buchanan, J. M.: Specific binding of azaserine with an enzyme of purine biosynthesis, *in* Abstracts of the 136th meeting, American Chemical Society, Boston, 1959, p. 45C.
80. Hsia, D. Y. -Y.: Inborn errors of metabolism, Chicago, 1959, Year Book Publishers, Inc.
81. Huennekens, F. M., and Osborn, M. J.: Folic acid coenzymes and one-carbon metabolism, Advances Enzymol. **21**:369-446, 1959.
82. Hutchison, D. J.: Biological activities of 6-mercaptopurine: Effects on *Streptococcus faecalis,* Ann. New York Acad. Sc. **60**:212-219, 1954.
83. Hutchison, D. J.: Metabolic and nutritional variations in drug-resistant *Streptococcus faecalis, in* Rebuck, J. W., Bethell, F. H., and Monto, R. W., editors: The leukemias: Etiology, pathophysiology, and treatment, New York, 1957, Academic Press, Inc., pp. 605-615.
84. Hutchison, D. J.: Metabolism of resistant mutants of *Streptococcus faecalis.* I. Isolation and characterization of the mutants, Cancer Res. **18**:214-219, 1958.
85. Hutchison, D. J., and Burchenal, J. H.: Studies on pteridine metabolism, *in* Wolstenholme, G. E. W., and O'Connor, C. M., editors: Ciba Foundation symposium on chemistry and biology of pteridines, Boston, 1954, Little, Brown & Company, pp. 366-380.
86. Jacquez, J. A., and Hutchison, D. J.: Resistance in L1210 ascites without change in concentrative uptake of O-diazoacetyl-L-serine or 6-diazo-5-oxo-L-norleucine, Cancer Res. **19**:397-401, 1959.
87. Jawetz, E.: Man's hand in bacterial resistance, CLIN. PHARMACOL. & THERAP. **1**:3-5, 1960.
88. Johnson, J. A., Jr., Hutchison, D. J., Galloway, R. E., and Skipper, H. E.: Studies on the mechanisms of resistance to temporary anticancer agents. I. Resistance to folic acid antagonists in *Streptococcus faecalis,* Proc. Am. A. Cancer Res. **2**:121-122, 1956.
89. Kalckar, H. M., Anderson, E. P., and Isselbacher, K. J.: Galactosemia, a congenital defect in a nucleotide transferase, Biochim. et biophys. acta **20**:262-268, 1956.
90. Kammen, H. O., and Hurlbert, R. B.: Amination of uridine nucleotides to cytidine nucleotides by soluble mammalian enzymes: Role

of glutamine and guanosine nucleotides, Biochim. et biophys. acta **30**:195-196, 1958.

91. Kammen, H. O., and Hurlbert, R. B.: The formation of cytidine nucleotides and RNA cytosine from orotic acid by the Novikoff tumor in vitro, Cancer Res. **19**:654-663, 1959.
92. Karnofsky, D. A.: Differences between cancers in terms of therapeutic responses, Cancer Res. **16**:684-697, 1956.
93. Kidder, G. W., Dewey, V. C., Parks, R. E., Jr., and Woodside, G. L.: Purine metabolism in tetrahymena and its relation to malignant cells in mice, Science **109**:511-514, 1949.
94. Korn, E. D., Remy, C. N., Wasilejko, H. C., and Buchanan, J. M.: Biosynthesis of the purines. VII. Synthesis of nucleotides from bases by partially purified enzymes, J. Biol. Chem. **217**:875-883, 1955.
95. Kornberg, A., Lieberman, I., and Simms, E. S.: Enzymatic synthesis of purine nucleotides, J. Biol. Chem. **215**:417-427, 1955.
96. Law, L. W.: Origin of the resistance of leukemic cells to folic acid antagonists, Nature, London **169**:628-629, 1952.
97. Law, L. W.: Effects of combinations of antileukemic agents on acute lymphocytic leukemia of mice, Cancer Res. **12**:871-878, 1952.
98. Law, L. W.: Resistance in leukemic cells to an adenine antagonist, 6-mercaptopurine, Proc. Soc. Exper. Biol. & Med. **84**:409-412, 1953.
99. Law, L. W.: Differences between cancers in terms of evolution of drug resistance, Cancer Res. **16**:698-716, 1956.
100. Law, L. W.: Some aspects of drug resistance in neoplasms, Ann. New York Acad. Sc. **71**: 976-992, 1958.
101. Law, L. W., and Boyle, P. J.: Development of resistance to folic acid antagonists in a transplantable lymphoid leukemia, Proc. Soc. Exper. Biol. & Med. **74**:599-602, 1950.
102. Law, L. W., Taormina, V., and Boyle, P. J.: Response of acute lymphocytic leukemias to the purine antagonist 6-mercaptopurine, Ann. New York Acad. Sc. **60**:244-250, 1954.
103. LePage, G. A., and Greenlees, J. L.: Incorporation of glycine-2-$C^{14}$ into ascites tumor-cell purines as a biological test system, *in* Gellhorn, A., and Hirschberg, E., editors: Investigation of diverse systems for cancer chemotherapy screening, Cancer Res. **15**: suppl. 3:102-105, 1955.

103a. LePage, G. A.: Symposium on the experimental pharmacology and clinical use of antimetabolites. V. Use of combinations of antimetabolites for chemotherapy of cancer, CLIN. PHARMACOL. & THERAP. **2**:121-129, 1961.

104. Levenberg, B., and Buchanan, J. M.: Biosynthesis of the purines. XIII. Structure, Enzymatic synthesis, and metabolism of (α-N-formyl)-glycinamidine ribotide, J. Biol. Chem. **224**:1019-1027, 1957.
105. Levenberg, B., Melnick, I., and Buchanan, J. M.: Biosynthesis of the purines. XV. The effect of aza-L-serine and 6-diazo-5-oxo-L-norleucine on inosinic acid biosynthesis de novo, J. Biol. Chem. **225**:163-176, 1957.
106. Liebicq, C., and Peters, R. A.: The toxicity of fluoroacetate and the tricarboxylic acid cycle, Biochim. et biophys. acta **3**:215-230, 1949.
107. Lindner, A.: Cytochemical effects of 5-fluorouracil on sensitive and resistant Ehrlich ascites tumor cells, Cancer Res. **19**:189-194, 1959.
108. Lukens, L. N., and Herrington, K. A.: Enzymic formation of 6-mercaptopurine ribotide, Biochim. et biophys. acta **24**:432-433, 1957.
109. Maas, W. K., and Davis, B. D.: Production of an altered pantothenate-synthesizing enzyme by a temperature-sensitive mutant of *Escherichia coli*, Proc. Nat. Acad. Sc. **38**: 785-797, 1952.
110. Mandel, H. G.: Effect of 8-azaguanine on utilization of methionine by *B. cereus*, Arch. biochem. et biophys. **76**:230-232, 1958.
111. Mandel, H. G.: The physiological disposition of some anticancer agents, Pharmacol. Rev. **11**:743-838, 1959.
112. Mandel, H. G., and Law, L. W.: The effect of 4-amino-5-imidazole-carboxamide on the carcinostatic action of 8-azaguanine, Cancer Res. **14**:808-811, 1954.
113. Mandel, H. G., and Altman, R. L.: The depression of the incorporation of sulfur amino acids into *Bacillus cereus* by 8-azaguanine, J. Biol. Chem. **235**:2029-2035, 1960.
114. Mandel, H. G., and Roodyn, D. B.: Differential effect of 8-azaguanine on cell wall and protoplasmic protein synthesis in *Bacillus cereus*, J. Biol. Chem. **235**:2036-2044, 1960.
115. Martius, C.: Über die Unterbrechung des Citronensäure-cyklus durch Fluoroessigsäure, Annalen **561**:227-232, 1949.
116. Matthews, R. E. F.: Biosynthetic incorporation of metabolite analogues, Pharmacol. Rev. **10**:359-406, 1958.
117. Miller, R. W., Lukens, L. N., and Buchanan, J. M.: The enzymatic cleavage of N-(5-amino- 1 -ribosyl- 4 -imidazolylcarbonyl)- L -aspartic acid 5′-phosphate, J. Biol. Chem. **234**:1806-1811, 1959.
118. Miner, R. W., editor: 6-Mercaptopurine, Ann. New York Acad. Sc. **60**:183-508, 1954.
119. Mitchell, J. H., Jr., Skipper, H. E., and Bennett, L. L., Jr.: Investigations of the nucleic acids of viscera and tumor tissue from animals injected with radioactive 8-azaguanine, Cancer Res. **10**:647-649, 1950.

120. Montgomery, J. A.: The relation of anticancer activity to chemical structure, Cancer Res. **19**:447-463, 1959.
121. Moore, E. C., and LePage, G. A.: In vivo sensitivity of normal and neoplastic tissues to azaserine, Cancer Res. **17**:804-808, 1957.
122. Moore, E. C., and LePage, G. A.: The metabolism of 6-thioguanine in normal and neoplastic tissues, Cancer Res. **18**:1075-1083, 1958.
123. Mukherjee, K. L., and Heidelberger, C.: Studies on fluorinated pyrimidines. IX. The degradation of 5-fluorouracil-6-$C^{14}$, J. Biol. Chem. **235**:433-437, 1960.
124. Nichol, C. A.: Studies on the mechanism of resistance to folic acid analogues in a strain of *Streptococcus faecalis*, J. Biol. Chem. **207**: 725-732, 1954.
125. Nichol, C. A.: Studies on resistance to folic acid antagonists, *in* Rebuck, J. W., Bethell, F. H., and Monto, R. W., editors: The leukemias: Etiology, pathophysiology and treatment, New York, 1957, Academic Press, Inc., pp. 583-604.
126. Nichol, C. A., and Welch, A. D.: On the mechanism of action of aminopterin, Proc. Soc. Exper. Biol. & Med. **74**:403-411, 1950.
127. Nichol, C. A., and Welch, A. D.: Metabolic requirements for formation of citrovorum factor and studies of mechanism of resistance to amethopterin, *in* Rhoads, C. P., editor: Antimetabolites and cancer, Washington, 1955, American Association for the Advancement of Science, pp. 63-87.
127a. Osborn, M. J., Freeman, M., and Huennekens, F. M.: Inhibition of dihydrofolic reductase by aminopterin and amethopterin, Proc. Soc. Exper. Biol. & Med. **97**:429-431, 1958.
128. Paterson, A. R. P.: The formation of 6-mercaptopurine riboside phosphate in ascites tumor cells, Canad. J. Biochem. & Physiol. **37**: 1011-1023, 1959.
129. Peters, R. A.: Lethal synthesis, Proc. Roy. Soc. **139**:suppl. B:143-170, 1952.
130. Pine, E. K.: Concentrative uptake of azaserine by neoplastic plasma cells and lymphocytes, J. Nat. Cancer Inst. **21**:973-984, 1958.
131. Potter, M.: Biologic studies on the development of DON resistance in a mast-cell neoplasm of the mouse, J. Nat. Cancer Inst. **23**: 163-181, 1959.
132. Potter, M., and Law, L. W.: Studies of plasma cell neoplasms of the mouse. I. Characterization of neoplasm 70429, including its sensitivity to various antimetabolites with the rapid development of resistance to azaserine, DON and N-methylformamide, J. Nat. Cancer Inst. **18**:413, 1957.
133. Potter, V. R.: Sequential blocking of metabolic pathways in vivo, Proc. Soc. Exper. Biol. & Med. **76**:41-46, 1951.
134. Reichard, P.: Enzymic synthesis of pyrimidines, Advances Enzymol. **21**:263-294, 1959.
135. Reichard, P., Sköld, O., and Klein, G.: Possible enzymic mechanism for the development of resistance against fluorouracil in ascites tumors, Nature, London **183**:939-941, 1959.
136. Roblin, R. O., Jr., Lampen, J. O., English, J. P., Cole, Q. P., and Vaughan, J. R., Jr.: Studies in chemotherapy. VIII. Methionine and purine antagonists and their relation to the sulfonamides, J. Am. Chem. Soc. **67**:290-294, 1945.
137. Salser, J. S., and Balis, M. E.: Studies on the mechanism of action of 6-mercaptopurine (6-MP) in cell-free preparations, Fed. Proc. **18**: 315, 1959.
138. Salser, J. S., Hutchison, D. J., and Balis, M. E.: Studies on the mechanism of action of 6-mercaptopurine in cell-free preparations, J. Biol. Chem. **235**:429-432, 1960.
139. Sartorelli, A. C., and LePage, G. A.: The development and biochemical characterization of resistance to azaserine in a TA3 ascites carcinoma, Cancer Res. **18**:457-463, 1958.
140. Sartorelli, A. C., and LePage, G. A.: Metabolic effects of 6-thioguanine. II. Biosynthesis of nucleic acid purines in vivo and in vitro, Cancer Res. **18**:1329-1335, 1958.
141. Sartorelli, A. C., LePage, G. A., and Moore, E. C.: Metabolic effects of 6-thioguanine. I. Studies on thioguanine-resistant and -sensitive Ehrlich ascites cells, Cancer Res. **18**:1232-1239, 1958.
142. Skipper, H. E.: On the mechanism of action of 6-mercaptopurine, Ann. New York Acad. Sc. **60**:315-321, 1954.
143. Skipper, H. E., Bennett, L. L., Jr., and Schabel, F. M., Jr.: Mechanism of action of azaserine, Fed. Proc. **13**:298, 1954.
144. Skipper, H. E., Mitchell, J. H., Jr., and Bennett, L. L., Jr.: Inhibition of nucleic acid synthesis by folic acid antagonists, Cancer Res. **10**:510-512, 1950.
145. Skipper, H. E., Montgomery, J. A., Thomson, J. R., and Schabel, F. M., Jr.: Structure-activity relationships and cross-resistance observed on evaluation of a series of purine analogs against experimental neoplasms, Cancer Res. **19**:425-437, 1959.
146. Skipper, H. E., Robins, R. K., Thomson, J. R., Cheng, C. C., Brockman, R. W., and Schabel, F. M., Jr.: Structure-activity relationships observed on screening a series of pyrazolopyrimidines against experimental neoplasms, Cancer Res. **17**:579-596, 1957.
147. Skipper, H. E., Schabel, F. M., Jr., Bell, M., Thomson, J. R., and Johnson, S.: On the

curability of experimental neoplasms. I. Amethopterin and mouse leukemias, Cancer Res. **17**:717-726, 1957.

148. Skipper, H. E., and Thomson, J. R.: Effects of a series of tumor-inhibiting agents and related compounds on L1210 leukemia and drug-resistant lines thereof, *in* Gellhorn, A., and Hirschberg, E., editors: Investigation of diverse systems for cancer chemotherapy screening, Cancer Res. **15**:suppl. 3:44-46, 1955.
149. Skipper, H. E., Thomson, J. R., and Bell, M.: Attempts at dual blocking of biochemical events in cancer chemotherapy, Cancer Res. **14**:503-507, 1954.
150. Stock, C. C., Reilly, H. C., Buckley, S. M., Clarke, D. M., and Rhoads, C. P.: Azaserine, a new tumor-inhibitory substance: Studies with Crocker mouse sarcoma 180, Nature, London **173**:71-72, 1954.
151. Szybalski, W.: Genetics of human cell lines. I. 8-Azaguanine resistance, a selective "single-step" marker, Proc. Soc. Exper. Biol. & Med. **101**:662-666, 1959.
152. Tomisek, A. J., Kelly, H. J., and Skipper, H. E.: Chromatographic studies of purine metabolism. I. The effect of azaserine on purine biosynthesis in *E. coli* using various $C^{14}$-labeled precursors, Arch. Biochem. & Biophys. **64**:437-455, 1956.
153. Tomizawa, S., and Aronow, L.: Studies on drug resistance in mammalian cells. II. 6-mercaptopurine resistance in mouse fibroblasts, J. Pharmacol. & Exper. Therap. **128**: 107-114, 1960.
154. Totter, J. R.: Antimetabolite studies on bone marrow in vitro, *in* Rhoads, C. P., editor: Antimetabolites and cancer, Washington, 1955, American Association for the Advancement of Science, pp. 153-162.
155. Tréfouel, J., Nitti, F., and Bovet, D.: Action of *p*-aminophenylsulfamide in experimental streptococcus infections of mice and rabbits, Compt. rend. Soc. biol. **120**:756-758, 1935.
156. Uhlenhuth, H.: Demonstration von mit Atoxyl behandelten Dourinekaninchen, Deutsche. med. Wchnschr. **33**:1237, 1907.
157. Wacker, A., Ebert, M., and Kolm, H.: Über den Stoffwechsel der *p*-Aminobenzoesäure, Folsäure und Aminofolsäure bei Enterococcus, Ztschr. Naturforsch. **13B**:141-147, 1958.
158. Way, J. L., and Parks, R. E., Jr.: Enzymatic synthesis of nucleotides of purine analogs, Fed. Proc. **16**:344, 1957.
159. Way, J. L., and Parks, R. E., Jr.: Enzymatic synthesis of 5′-phosphate nucleotides of purine analogues, J. Biol. Chem. **231**:467-480, 1958.
160. Welch, A. D.: The problem of drug resistance in cancer chemotherapy, Cancer Res. **19**:359-371, 1959.
161. Woodruff, H. B., and McDaniel, L. E.: The antibiotic approach, *in* Cowan, S. T., and Rowatt, E., editors: The Strategy of chemotherapy, London, 1958, Cambridge University Press, pp. 29-48.
162. Zakrewski, S. F., and Nichol, C. A.: On the enzymic reduction of folic acid by a purified hydrogenase, Biochim. et biophys. acta **27**: 425-426, 1958.

*(To be continued)*

# Nerve injury from intramuscular barbiturate injection

*Intramuscular injection of drugs is usually considered a safe procedure. Occasionally, however, nerve damage, particularly to the sciatic nerve, has been reported following various drugs. There has been some dispute regarding the role which the chemical nature of the drug, the simple physical pressure effect, and technique play in such incidents.*

*A case is reported in which definite sensory damage to the sciatic nerve occurred in a patient after the intragluteal administration of amobarbital sodium. Experiments were undertaken in which amobarbital sodium solution was injected along the course of the sciatic nerve in guinea pigs. Hypalgesia and decrease of muscle tone developed in all instances, and in some of the animals trophic changes of the feet appeared. The need for careful technique in placing the intramuscular injection outside the course of the sciatic nerve is discussed.*

**Richard K. Richards, M.D.** *Chicago, Ill.*
*Department of Pharmacology, Northwestern University Medical School*

Intramuscular injection is a commonly used and, in general, a safe method of drug administration. Occasionally, though, nerve damage has followed intragluteal injection. Faulty technique, the chemical nature of the medication, or pressure effects have been judged responsible.

A case of damage to the sciatic nerve after amobarbital sodium injection came to our attention recently. A search of the literature unearthed only two reports of this type with an injectable barbiturate. Keown and Hitchcock[4] reported 1 case of damage to the sciatic nerve among 50 children injected with 2.5 ml. of a 5 per cent solution of thiopental sodium; complete recovery followed after several months. In another paper, Keown and associates[3] warn to carefully avoid the area of the sciatic nerve for such injections because temporary or even permanent muscular paralysis may result. Lieberman[5] reported a case of severe nerve damage following extravasal injection of thiopental sodium (concentration not stated) into the cubital fossa. Anesthesia developed in the area supplied by the nerve running through this region; grip strength was reduced for 10 months. Wicks and Livingstone[9] mention subcutaneous irritation but no damage after the intramuscular injection of thiopental sodium. No report of nerve damage following intramuscular administration of amobarbital or pentobarbital was found.

## Case report

A 60-year-old white man, whose history included mild, controlled diabetes and a subtotal gastrectomy for a benign polyp of the stomach, was hospitalized because of postoperative adhesions. The patient had suffered a shrapnel in

Received for publication Dec. 5, 1960.

jury to his left leg just above the lateral aspect of the knee in the First World War. This resulted in a foot drop with loss of function of the ankle, clawing of the toes, and some muscular atrophy. He wore a foot brace and walked with a limp but usually without a cane. He was able to perform fairly heavy physical work without marked impairment and without pain.

Following the operation for adhesions amobarbital sodium in quantities of 250 mg. dissolved in 2.5 ml. water was injected repeatedly intramuscularly into the buttocks by a nurse. Following one of such injections, the patient complained of numbness in the foot on the injected (left) side which subsided somewhat after massage. Examination by the physician indicated the presence of a needle puncture over the course of the sciatic nerve, midway between the greater trochanter and the ischial tuberosity. Pinpricks were felt in the area of numbness. A diagnosis of toxic neuritis was made.

After discharge, the patient experienced severe pain and paresthesia along the lower left limb. He was readmitted 1 month later, at which time he needed assistance when walking because of pain and weakness.

Repeated neurologic examination was somewhat complicated by the pre-existent war injury, especially that to the left popliteal nerve. However, definite sensory changes on the medial aspect of the left foot with apparently unimpaired muscular strength could not be related to the old injury and were compatible with chemical injury to the nerve fibers caused by the amobarbital sodium injection, with the subjective complaints of the patient, and with his difficulties in walking. Neurologists expressed the opinion that the sensory fibers would ultimately regenerate.

### Experimental studies

It appeared profitable to provide further information by undertaking experiments, closely simulating clinical conditions. We have been unable to find experimental studies of this type in the literature.

For this purpose, a number of experiments were conducted, using a 10 per cent aqueous solution of amobarbital sodium. The pH of such solutions is approximately 9.5, and 1.8 ml. of 0.1N HCl per milliliter of drug is necessary to reach a pH of approximately 7, indicating a fair buffering capacity of this barbiturate.

Two guinea pigs of about 300 Gm. weight were used in the first experiment. Deep intramuscular injections were given into the ridge between the greater trochanter and the ischial tuberosity on the left side of 0.2 ml. of a 10 per cent amobarbital sodium solution, following the technique described by Shackell[8] for producing sciatic nerve block with local anesthetics. An equal amount of saline was injected into the right side to control nonspecific pressure effects.

Beginning within 30 minutes after injection, a moderate but definite impairment of the withdrawal reflex could be demonstrated on the lateral aspects of the left lower limb in both animals, when tested with a mouth-tooth forceps. Though still able to walk, both animals placed the left foot into an awkward position of lateral abduction, especially when prodded into moving. When held by hand so that both legs hung freely, the left leg showed decreased tonus. These observations were interpreted as indicative of hypalgesia and diminution of tonus and muscle sense, without obvious impairment of gross muscular strength.

On repeated examination over the next 10 days, no significant change was noted. Beginning with the eleventh day after injection in one animal and the seventeenth day in the other animal, open sores developed on the left feet. These progressed over the next few weeks and healed finally after loss of most of the left foot in both.

Approximately 6 months later, the two animals were killed and the sciatic nerves excised. Sections were prepared and stained with hematoxylin and eosin, Kluver's Luxol fast blue with PAS, and Wilder's silver stain for reticulum. The nerves injected with saline appeared histologically normal. However, the two injected with amobarbital sodium showed scarring characterized by proliferation of the perineurium. Demyelinzation was demonstrated with the Kluver stain. There was also evidence of chronic inflammatory changes and round-cell infiltration* (Fig. 1).

The reproducibility of these results was ascertained with a second set of three

*R. J. Stein: Personal communication.

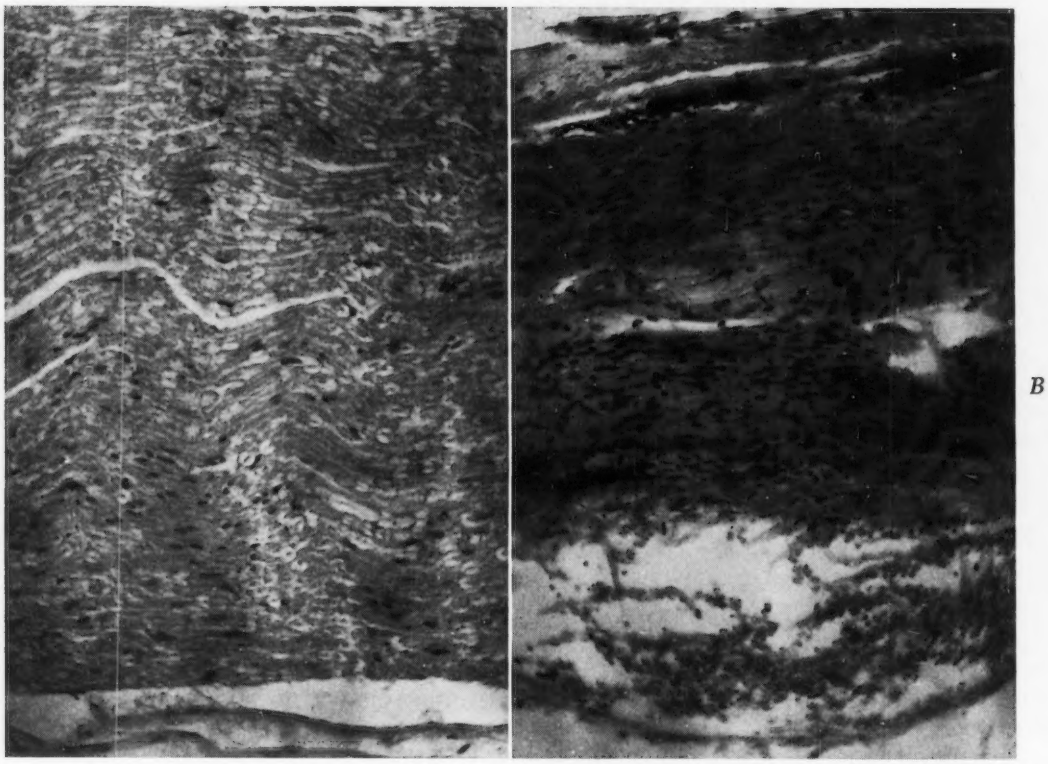



**Fig. 1.** *A*, Control: Guinea pig sciatic nerve after injection of normal saline. Note normal complement of myelin and normal histologic appearance of nerve fibers. *B*, Experimental: Guinea pig sciatic nerve after injection of 10 per cent amobarbital sodium. Marked demyelinization with secondary inflammatory reaction is evident. Hemorrhage and edema are also seen. (Hematoxylin and eosin ×400; reduced 4/9.)

guinea pigs. The same plan was followed as utilized in the first group except that 0.3 ml. of the 10 per cent amobarbital sodium solution was used on the left side and an equal amount of saline on the right side. Hypalgesia and loss of muscular coordination and tone on the left side were noticed almost immediately. With minor variations, these changes persisted for the 3 week observation period and appeared to stabilize at this level for a few additional weeks. After about 8 weeks, the findings returned gradually to normal.

## Discussion

The clinical case reported here and the experiments in guinea pigs provide evidence that intramuscular injection of a soluble barbiturate can produce nerve damage. This is in agreement with the report of Keown and Hitchcock[4] and with the result of paravenous injection of thiopental.[5] However, in previous reports of such complications, a 5 per cent solution of thiopental sodium, which has a pH of 10.5 and a titrable acidity of 2.1 ml. 0.1 N HCl per ml., was used, thus exceeding the alkalinity and buffering capacity of a 10 per cent amobarbital sodium solution. Five per cent solutions of the sodium salts of pentobarbital, secobarbital, barbital, and thiopental were studied as experimental spinal anesthetics in dogs without serious sequelae[7]; however, bladder dysfunction and hypalgesia have followed such procedures in man,[6] and they therefore appear to have been abandoned.

Because the possibility of severe nerve damage with soluble barbiturates is now established, the importance of anatomically correct intramuscular injection becomes clear. Von Hochstetter[2] collected data on

91 cases of nerve paralysis over 5 years following intramuscular injection with various drugs; pressure of the solution alone seemed rarely to be responsible. Damage resulted more often with poorly absorbed drugs. In most instances, epineural or paraneural rather than intraneural injections seemed to have been given. In such cases, pain or paralysis may occur only after a symptomless interval. On the basis of careful anatomic dissection, von Hochstetter concluded that the usual recommendation for injection into the upper outer quadrant of the gluteal region is too vague and does not preclude placement of the drug into or close to the sciatic nerve. He recommends a much more lateral site. The original article should be consulted for the details.* These proposals have recently been corroborated by Curtiss and Tucker,[1] who interpreted partial sciatic paralysis in 10 premature infants as sequelae of gluteal injections with antibiotics and other agents.

### *References*

1. Curtiss, P. H., Jr., and Tucker, H. J.: Sciatic palsy in premature infants, J.A.M.A. **174**:1586-1588, 1960.
2. von Hochstetter, A.: Ueber Probleme und Technik der intragluteal Injektion: Der Einfluss des Medikamentes und der Individualitat des Patienten auf die Entstehung von Spritzenschaden. I, Schweiz. med. Wchnschr. **85**:1138-1144, 1955; II, Schweiz. med. Wchnschr. **86**: 69-76, 1956.
3. Keown, K. K., Fischer, S. M., Downing, D. F., and Hitchcock, P.: Anesthesia for cardiac catheterization in infants and children, Anesthesiology **18**:27-274, 1957.
4. Keown, K. K., and Hitchcock, P.: Intramuscular injections of Pentothal sodium, Anesth. & Analg. **39**:1-6, 1960.
5. Lieberman, S. L.: Nerve destruction following extravascular injection of Pentothal sodium, Anesthesiology **7**:676, 1946.
6. Morrison, R. L., Koppanyi, T., and Tuohy, E. B.: The spinal anesthetic effects of barbiturates, Anesthesiology **12**:315-320, 1951.
7. Purdy, F. A., and Westfall, B. A.: Barbiturates as spinal anesthetics (comparison of barbital, pentobarbital, secobarbital, thiopental with procaine as spinal anesthetics in dogs), J. Pharmacol. & Exper. Therap. **118**:318-321, 1956.
8. Shackell, L. F.: Tests of local anesthetics by sciatic nerve block in the intact guinea pig, Anesth. & Analg. **14**:20-22, 1935.
9. Wicks, W. R., and Livingstone, H. M.: Intramuscular Pentothal sodium and benzyl alcohol as a pre-anesthetic agent, Anesth. & Analg. **25**: 204-207, 1946.

*Injections into the vastus lateralis muscle are now being proposed by British authors (Bayliss, R. J. S.: Practical procedures in clinical medicine, London, 1954, J. & A. Churchill, Ltd., p. 17).

# New information on drugs

## Excerpt from the *Federal Register*

**Changes in Drug-Labeling Requirement; Changes in New-Drug Applications; Amendment in Final Order and Changes in Effective Date**

On December 9, 1960, the Commissioner of Food and Drugs, pursuant to section 701 of the Federal Food, Drug, and Cosmetic Act and under the authority delegated to him by the Secretary of Health, Education, and Welfare (25 F.R. 8625) published in the FEDERAL REGISTER (25 F.R. 12592) the final order amending the regulations for the enforcement of the drug and device sections and the new-drug section of the law for the purposes of requiring manufacturers to furnish adequate information for the professional use of prescription drugs and devices and to keep new drugs off the market until the adequacy of manufacturing methods, facilities, and controls have been confirmed by establishment inspection, when required.

Portions of the order were to become effective on January 8, 1961, and other portions were scheduled to become effective on March 9, 1961.

Representatives of the industries involved have demonstrated to the Commissioner that it is impracticable to effect full compliance with those portions of the order scheduled to become effective on January 8, 1961, by that date. Additionally, the Commissioner concludes that certain portions of the published regulations require clarifying revisions.

*Therefore, it is ordered:*

1. That the requirements of § 1.106(b) (2) (ii), (iii), (iv), and (v) and 1.106(c) (ii), (iii), (iv), and (v) shall not, until January 1, 1962, be applicable to drugs bearing labels printed prior to January 8, 1961, if the information required by these sections is contained in the labeling within the package from which the drugs are to be dispensed.

2. That the requirements of § 1.106(b) (2) (vi) and 1.106(c) (2) (vi) shall not be applicable, until June 6, 1961, to any carton of a drug packaged prior to January 8, 1961, if the identifying lot or control number appears on the label of the drug contained in that carton.

3. That the provisions of § 1.106(b) (4), (c) (4), and (d) (4) shall not apply to catalogs and price lists distributed to pharmacists and wholesale druggists (but not to physicians or other practitioners), until January 1, 1962.

---

Reprinted in full from vol. 26, no. 9, 1961.

4. Section 1.106 *Drugs and devices; directions for use* is amended in the following respects:

a. Paragraph (b) (2) (v) is amended to read as follows:

(v) If it is for other than oral use, the names of all inactive ingredients, except that:

(*a*) Flavorings and perfumes may be designated as such without naming their components.

(*b*) Color additives may be designated as coloring without naming specific color components unless the naming of such components is required by a color additive regulation prescribed in Part 8 of this chapter.

(*c*) Trace amounts of harmless substances added solely for individual product identification need not be named.

(vi) If it is intended for administration by parenteral injection, the quantity or proportion of all inactive ingredients, except that ingredients added to adjust the pH or to make the drug isotonic may be declared by name and a statement of their effect; and if the vehicle is water for injection it need not be named.

b. Paragraph (c) (2) (v) is amended to read as follows:

(v) If it is for other than oral use, the names of all inactive ingredients, except that:

(*a*) Flavorings and perfumes may be designated as such without naming their components.

(*b*) Color additives may be designated as coloring without naming specific color components unless the naming of such components is required by a color additive regulation prescribed in Part 8 of this chapter.

(*c*) Trace amounts of harmless substances added solely for individual product identification need not be named.

(vi) If it is intended for administration by parenteral injection, the quantity or proportion of all inactive ingredients, except that ingredients added to adjust the pH or to make the drug isotonic may be declared by name and a statement of their effect; and if the vehicle is water for injection, it need not be named.

c. Paragraphs (b) (4) and (c) (4) are amended by inserting in the first sentence, just before the last word "contain," the parenthetical phrase "(other than dose information required by § 1.106 (b) (2) (ii) and (c) (2) (ii) )".

5. That the effective date for the amendments published December 9, 1960, scheduled to become effective on January 8, 1961, is hereby changed to February 7, 1961.

Notice and public procedure are not necessary prerequisites to the promulgation of this order since the changes in effective dates and other amendments relax existing requirements.

*Effective date.* This order shall be effective on the date of signature.

Dated: January 6, 1961.

[SEAL] JOHN L. HARVEY,
*Deputy Commissioner of Food and Drugs.*

## Book reviews

**The Central Nervous System and Behavior,** edited by M. A. B. Brazier. New York, 1959, Josiah Macy, Jr. Foundation. 358 pages. $4.75.

Merits and demerits to publish the kind of group discussion which has become so characteristic of the Josiah Macy, Jr. Foundation symposia have been pointed out by many reviewers in the past. Whether one likes or dislikes this kind of publication is, I believe, strictly dependent on what kind of "information" one wishes to obtain. I doubt whether we always mean the same when we talk about "scientific information" in different contexts. Yet, it is the interpretation of this concept which determines our evaluation of the publication of essentially unedited group conferences. "Scientific information" is being frequently identified with "bits of information," that is, with a magnitude subject to measurement by the communication engineer. To those who view scientific information as something that can be "collected, organized, and distributed," publication of group conferences of this type should, I believe, appear unappropriate. Against this, I hold that such interpretation of "scientific information" fails to recognize the active role of the "receiver" to endow factual statements with meaning, i.e., to relate them to a specific context. It is in this area in which creativity, originality, and the personal element of "knowing" come into their right. And it would be in this area that the printed record of a group conference accomplishes a purpose, unattainable by traditional forms of publications, in that it acquaints the reader with the personal attitude of different people toward a problem or a piece of evidence. In fact, I contend that it is the very recognition of the personal element in scientific judgments which justifies the publication of unedited group conferences and which gives them a special place among scientific publications.

How many fold the approaches to the central nervous system and behavior are, and how the responses to specific observations vary within a group of people with different background and interests, is revealingly illustrated by the proceedings of this symposium.

In a presentation on the limbic system, P. D. MacLean pays tribute to the pioneering efforts of Papez in identifying the limbic cortex as the neural substrate for the elaboration of emotional reactions.

Pleasure reactions, grooming, and sexual manifestations induced by electrical or chemical stimulation of the hippocampus constitute the central theme of the work presented by MacLean. The discussants contribute to this presentation mainly in the field of self-stimulation (J. Olds), and a case is forcefully made for a common denominator of the hypothalamic-midbrain-limbic region; while the nature of elicited responses may differ greatly in different zones of that system, almost all of that system affords positive reinforcement of behavior on stimulation. MacLean's suggestion that the limbic system is involved in euphoria and psychosis is discussed in view of its responsiveness to a variety of excitatory, sedative, and analgesic drugs.

Electrical activity in the hippocampus in relation to behavioral responses has been for some time under close investigation by E. Grastyan, K. Lissak, and others. Grastyan describes authoritatively in this symposium the hippocampal electric activity in the course of conditioning procedures. Experiments of M. E. Olds support Grastyan's contention that midline systems in the hippocampus (and thalamus) are peculiarly involved in the learning activity, but these structures do not seem important for correct performance after learning has occurred. This finding does not conflict with, and possibly supports, Grastyan's earlier claim that attention may be attributed to hippocampal structures. Promising perspectives are explored by the discussants, e.g., hippocampal inhibition of intrinsic conduction in the reticular formation (Livingston) and detailed synaptic organization of the archicortex (Purpura).

Electroencephalographic correlates of conditioned reflex formation in man are discussed by V. S. Rusinov; these findings are compared by the discussants with relevant work in animals. Most appropriately, the conceptual difficulties in this area are pointed out by R. B. Livingston and attributed to our language structure, which channels relations between events into a linear cause-effect mold, a relationship which is unfit for representation of the multiple interdependences encountered in complex neuronal structures.

The usefulness of "spreading depression" to establish a reversible, functional decortication is explored by J. Burês. In particular, the relation of this state to hippocampal electrical activity is described.

In a summation, R. Galambos crystallizes succinctly the key issues of this symposium's agenda, reduces them to their essentials, and exposes with uncomprising precision the fundamentals of the conditioned and the unconditioned response. The present volume also contains a vivid report by M. Brazier on the Moscow Colloquium on EEG and Higher Nervous Activity, held in 1958. A list of translations of Russian publications, compiled by the Department of Health, Education, and Welfare, U. S. Public Health Service, and made available in a volume entitled "The Central Nervous System and Behavior," is also included.

*Gerhard Werner*

**Detoxication Mechanisms,** second edition, by R. T. Williams. London, 1959, Chapman & Hall, Ltd. 796 pages. $18.00.

I had never read the first edition of *Detoxication Mechanisms,* so my reading of the present edition came as a new experience along with a shocking realization that for 12 years I had missed an important and useful text. The book is an extraordinary storehouse of valuable information on the metabolism and detoxication of drugs, toxic substances, and other organic compounds.

The author wastes little time on generalities. After a brief introduction, he plunges right into the intimate details of the detoxication mechanisms and metabolic pathways of an all-inclusive list of drugs ranging from the simple alcohols and aliphatic compounds up the scale through the

complex heterocyclic compounds. The old as well as the very latest drugs are included. The organometallic compounds are rather briefly discussed. The final chapter, which is a short one, has a brief account of some hypotheses which have been put forward to explain the need for the detoxication of foreign materials and the author's explanation of their shortcomings. There is a very short statement on biochemically inert compounds.

The book is a wonderful compilation of highly specialized information. The organization is excellent, the writing clear, precise, and concise. Despite its large size, it would be too much to expect extensive discussions of particular points in such a work; this deficiency is compensated for by adequate lists of references which follow each chapter. All combine to make this the outstanding source book on the subject; I know of no other like it. I do not see how any modern medical library, pharmacologist, biochemist, or physiologist can afford not to have a copy available.

*Walter Modell*

**Selective Toxicity,** second edition, by A. Albert. New York, 1960, John Wiley & Sons, Inc. 233 pages. $5.50.

To those who would like to see pharmacology more generally appreciated as a basic science *sui generis*, it is distressing that very few current monographs discuss explicitly and exclusively the working concepts, explanatory principles, and basic ideas which constitute the descriptive order of, and impart predictive value to, statements on drug actions. Professor Adrien Albert's book *Selective Toxicity,* the first edition of which appeared in 1951, fulfilled the important task of systematically relating drug specificity to chemical and physicochemical properties of agents and, thereby, covered most successfully one of the several approaches in basic pharmacology.

While the present, second edition essentially follows the pattern set by the earlier version, some important changes were introduced. Apart from some rearrangement of and addition to the material presented in the first edition, two additional topics are discussed, one dealing with absorption, distribution, and elimination of drugs, the second with pharmacodynamics.

In spite of these additions, the emphasis of the monograph remains unquestionably in the field of the chemical basis for drug selectivity. The principle of metabolite antagonism, the role of ionization in drug action, the Ferguson effect, and the role of covalent bond formation in drug action are lucidly presented and illustrated by well-chosen examples, many selected from Professor Albert's own work. To this list of topics can be added the consideration of steric factors and surface chemistry. Considerable attention is directed to metal-binding agents, to the effect that their biologic significance emerges very clearly; while stability constants and other physicochemical properties of chelates are discussed, it might have been useful in this section to add a few words on the peculiarity of coordination bonds.

It appears that Professor Albert felt a need in this edition to help the reader in bridging the gap between the study of drug action on effector cells in isolation and in intact organisms. To this purpose, a brief discussion of the factors governing drug transport to, and elimination from, effector sites was included. This discussion is sketchy and does not more than touch upon the most general principles. While this discussion undoubtedly will be helpful to the uninitiated, it may also, to some extent, mislead by not sufficiently stressing the complexity of events encountered in drug transport and elimination.

The chapter on pharmacodynamics does not give more than a bird's eye view of the description of drug action in terms of physiologic functions affected by chemical agents. While the principle of reducing complicated chemical compounds to essen-

tial, active configurations is well documented by examples, it is not, in my opinion, sufficiently stressed how planned design of chemical agents can serve as a significant tool in the elucidation of physiologic functions.

Admittedly, the chapters on pharmacodynamics and on drug transport and elimination are subsidiary to the main theme of the book, namely, the chemical basis of drug selectivity. Although in the latter area the present monograph most successfully fulfills the important task of bringing together in well-organized and lucid form the basic concepts of chemical pharmacology, it may endanger the unprepared reader to adopt too superficial a view on the former approaches in pharmacology.

*Gerhard Werner*

**The Dispensatory of the United States of America, 1960 Edition, New Drug Developments Volume,** edited by Arthur Osol and Robertson Pratt. Philadelphia, 1960, J. B. Lippincott Company. 240 pages. $9.00.

The rather long-winded title above means that this book forms Volume Two of U. S. D. 25, which was published in 1955, and provides information about the approximately 200 new drugs which have since come into use, as well as new data on old drugs.

As in U. S. D. 25, drugs are listed alphabetically according to generic name, and in addition, there is an index at the end of the book so that drugs can easily be found. Following the preface, there is a list of generic names which have appeared since 1955; some of these are the names of drugs which have until recently carried only a chemical label, and others have been changed to bring them into line with drugs of similar chemical composition and usage. Judging by the twenty-three new generic names in the list, they are relatively easy to pronounce and give some idea about the nature of the drug or its use, for example, glucosulfone sodium (Promin).

There are good survey articles on subjects such as the antidiabetic drugs and psychotherapeutic and psychotomimetic drugs. A great deal of space is devoted to antibiotics, and numerous references are quoted. However, space could have been saved by cutting down on many of the papers quoted, although in general a critical attitude is maintained. Occasionally one becomes really interested in a drug but is told to see a reference article, when more information could have been given at that point. At times the absence of a critical clinician on the staff becomes obvious, as, for example, in the case of dibenzylene hydrochloride (in U. S. D. 25): "When administered orally, and especially so when administered intravenously, the most common complaint of patients was nasal congestion. Dizziness, especially as associated with upright posture (orthostatic hypotension) is also a common complaint. Consequently, most patients are more comfortable when confined to bed during therapy."

It is inevitable that some inaccuracies creep into a book of this nature. For example, fumagillin is mentioned as useful in amebiasis, but the most recent paper quoted in support is dated 1955; few physicians with any practical experience with amebiasis will accept this. Glutethimide (Doriden) could not be found in either the text or the index of U. S. D. 25, Volumes 1 and 2. This is a surprising omission, since the drug is being used widely and cases of poisoning are now being reported.

The choice of a drug for a particular condition is now more difficult than ever, and this book does not really help, although a great deal of other information is available. References are remarkedly up to date, and many papers which appeared in 1960 are quoted—a very creditable performance. Unfortunately, by the time the next issue

of the U. S. D. appears, all these will probably be very much out of date.

One may think that reviewing a book of this nature is dull; on the contrary, it is most interesting and instructive. In particular, one is struck by the similarity of the structural formulas of many similar drugs (in particular the promazine and chlorothiazide group) with completely dissimilar names. However, Volume Two has one great advantage over U. S. D. 25, 1960: it weighs a mere 0.68 Kg. to the 4.2 Kg. of U. S. D. 25.

*Edel Berman*

**Toxicology: Mechanisms and Analytical Methods,** Volume I, edited by C. P. Stewart and A. Stolman. New York, 1960, Academic Press, Inc. 774 pages. $22.00.

The first volume of this two volume work is divided into two parts; the first treats absorption, distribution, and excretion of poisons and their metabolites, while the second discusses the principal general analytic procedures employed by the toxicologist.

In part one, toxic materials are divided into several classes depending on their physical and chemical properties (volatility, acidic or basic nature, metallic composition), and representative members of these classes are described. The number of individual substances discussed in this part is rather large. A fairly extensive bibliography is provided.

The discussion of general analytic procedures of use to the toxicologist is very complete and includes most of the newer analytic tools available. The first two chapters in this section cover the systematic search for an unknown poison in the viscera and isolation and separation of the various classes of poisons from biologic material. These are followed by the discussion of the various specialized analytic techniques which have proved useful in the isolation and identification of toxic materials. In this area, chromatographic methods, use of ion exchange resins and paper ionophoresis, countercurrent distribution, x-ray diffraction analysis, optical-crystallographic methods of drug identification, and a thorough study of spectrography as applied to toxicologic investigations are considered.

In general, this work is a competent treatment of isolation and identification of poisons from biologic material.

*Edmund J. Gaughan*

## Books received

Adriani, J.: The Pharmacology of Anesthetic Drugs, ed. 4, Springfield, Ill., 1960, Charles C Thomas, Publisher. 232 pages. $11.00.

Beecher, H. K., Editor: Disease and the Advancement of Basic Science, Cambridge, Mass., 1960, Harvard University Press. 416 pages. $12.50.

Daley, R., Goodwin, J. F., and Steiner, R. E., Editors: Clinical Disorders of the Pulmonary Circulation, Boston, 1960, Little, Brown & Company. 364 pages. $14.00.

Goodwin, T. W.: Recent Advances in Biochemistry, Boston, 1960, Little, Brown & Company. 301 pages. $11.50.

Greenberg, D. M., Editor: Metabolic Pathways, vol. 1, New York, 1960, Academic Press, Inc. 572 pages. $18.00.

Hilsenrath, J., and Coauthors: Tables of Thermodynamic & Transport Properties, New York, 1960, Pergamon Press, Inc. 478 pages. $20.00.

Meyler, L.: Side Effects of Drugs, Amsterdam, 1960, Excerpta Medica. 239 pages.

Michelfelder, W.: It's Cheaper to Die, New York, 1960, George Braziller, Inc. 192 pages. $3.50.

Moodie, W.: Hypnosis in Treatment, New York, 1960, Emerson Books, Inc. 168 pages. $4.00.

O'Brien, R. D.: Toxic Phosphorous Esters: Chemistry, Metabolism, and Biological Effects, New York, 1960, Academic Press, Inc. 434 pages. $14.50.

Osol, A., Farrar, G. E., Jr., and Pratt, R., Editors: The Dispensatory of the United States of America, ed. 25, Philadelphia, 1960, J. B. Lippincott Company. 2,379 pages. $30.00.

Polson, C. J., and Tattersall, R. N.: Clinical Toxicology, Philadelphia, 1961, J. B. Lippincott Company. 589 pages. $10.00.

Prigge, R., and Heymann, G.: Fundamentals and Possibilities in Anti-tuberculosis Vaccination, Toronto, 1960, University of Toronto Press. 105 pages. $5.00.

Rossier, P. H., Buhlmann, A. A., and Wiesinger, K. (Edited and translated from the German edition by Luchsinger, P. C., and Moser, K. M.): Respiration; Physiologic Principles and Their Clinical Applications, St. Louis, 1960, The C. V. Mosby Company. 505 pages.

Stewart, C. P., and Stolman, A., Editors: Toxicology: Mechanisms & Analytical Methods, vol. 1, New York, 1960, Academic Press, Inc. 774 pages. $22.00.

Symposium: Carbon Dioxide and Man, Anesthesiology, November-December, 1960. 77 pages. $2.00.

Wallis, T. E.: Textbook of Pharmacognosy, ed. 4, London, 1960, J. & A. Churchill, Ltd. (Distributed in the United States by Little, Brown & Company.) 640 pages.

Wilson, J. R.: The Double Blind, New York, 1960, Doubleday & Company, Inc. 286 pages. $3.95.

Wolstenholme, G. E. W., and O'Connor, M., Editors: Haemopoiesis: Cell Production and Its Regulation (Ciba Foundation Symposium), Boston, 1960, Little, Brown & Company. 490 pages. $11.00.

Wolstenholme, G. E. W., and O'Connor, C. M., Editors: Regulation of the Inorganic Ion Content of Cells (Ciba Foundation Study Group 5), Boston, 1960, Little, Brown & Company. 100 pages.

Wright, S. E.: The Metabolism of Cardiac Glycosides, Springfield, Ill., 1960, Charles C Thomas, Publisher. 86 pages. $4.75.

## Announcement

It is with great pleasure we announce that Dr. Hylan Bickerman of the Columbia University, College of Physicians and Surgeons, Dr. Harry F. Dowling of the University of Illinois, College of Medicine, Dr. Richard Kessler of Cornell University Medical College, Dr. R. K. Richards of Northwestern University Medical School, and Dr. C. Gordon Zubrod of the National Cancer Institute have joined the Editorial Board of CLINICAL PHARMACOLOGY AND THERAPEUTICS.

## Nomenclature of cancer chemotherapy agents

*This paper does not attempt to define all of the rules of nomenclature but only points out a few of the basic rules and gives examples of how they are used in naming compounds of interest as cancer agents.*

In everyday life, coined words, trade names, etc., have been adopted the purpose of which is to be concise yet imply composition or use. The names of organic chemicals are extremely long and very likely unpronounceable for the average person. To replace these long descriptive names, a shorter word may come into common usage.

The common name of a chemical is used for ease of reference. It is often derived from a part of the chemical name, as in stilbestrol, the complete name of which is $\alpha,\alpha$-diethyl-4, 4′-stilbenediol; or the common name may be designated by taking the first letters of the chemical name, for example, 6-AN for 6-*a*mino-*n*icotinamide; or a name may be composed by lifting out parts of the longer name, such as chloramine[1] for 2,2′-di*chloro*-N-methyldiethyl*amine* hydrochloride. Also, a name may imply its use, as in Placidyl (ethochlorvynol) for 1-chloro-3-ethyl-1-penten-4-yn-3-ol, a sedative. Sometimes a common name becomes so thoroughly entrenched by usage that it is adopted as a chemical name, e.g., testosterone rather than 17$\beta$-hydroxyandrost-4-en-3-one. Another source for a common name is the name of the plant from which the chemical is extracted, for example, gibberellic acid, a plant growth–promoting metabolite of *Gibberella fujikuroi.*[2]

"Man has handled chemical substances from the beginning of human activity and names given to common materials are, in many instances, as old as speech itself. They were coined as the need arose. Examples are water, iron, salt. With the development of metallurgy and medicine more and more varied materials came into use and required naming, and the haphazard method resulted in names that often were neither convenient nor sensible. With the rise and growth of alchemy there came a flood of new names, often picturesque, frequently absurd, and in many cases confusing by reason of a multiplicity of terms to designate the same substance. For instance, the material now called potassium sulfate had five names: sal polychrestum Glaseri, tartarus vitriolatus, vitriolum potassae, sal de duobus, arcanum duplicatum. Again such mouthfuls as powder of algaroth, sal alembroth, turbith mineral, colcothar, aethiops, are instances of names hard to remember and that give no information about the substances to which they were applied. A 'pelican' was not a bird but a distilling vessel, the 'death's head' was the head of an alembic, 'caput mortuum' or 'terra damnata' meant the residue from a distillation. Equally ridiculous were 'oil of vitriol,' 'butter of arsenic,' 'liver of sulfur,' 'sugar of lead,' 'flowers of zinc,' 'milk of magnesia,' which are instances of names that are actually misleading and, as Dumas remarked, give the impression that the chemists borrowed their language from the kitchen, and as Lavoisier pointed out, are positively dangerous because most of these substances are poisonous. Is it any wonder that only specialists, now or even then, could read understandingly the chemical treatises in which such a barbarous nomenclature was used?"[4]

The basic principles of our present system of nomenclature were developed in 1787 by a French chemist, Gayton de Morveau (1737-1816). He believed that the name of the chemical should convey the composition of the compound, but not by the use of a phrase or by the chemist's name (e.g., Glauber's salt) as was then the practice. The names should be coined from Latin or Greek so that their meanings would be more widely and easily understood. The evolution of the naming of organic compounds has been slowly developing since then. Our present system has come about through numerous conferences, both national and international. It was at Geneva in 1892 that thirty-four of the best qualified chemists of Europe laid the foundation for a system of nomenclature, but unfortunately the details were not worked out except for the acrylic compounds. The names proposed were too complicated and not practical, and this is probably the reason that the Commission of 1892 did not complete its work. The basic ideas worked out in Geneva have, however, aided the chemist in developing a system for naming organic compounds.

In 1922 the International Union of Chemistry named a committee to study the reform of organic nomenclature. Dr. E. J. Crane of Chemical Abstracts was the United States representative. He was replaced a year later by A. M. Patterson. This Committee held several meetings in Paris during the years 1924 to 1928. The result of its work was a report presented to the Union at The Hague in 1928. Some modifications which were made and presented to the Commission in 1930 at Liège were adopted unanimously. "The object of the Committee in its work has been rather to follow usage as nearly as possible, to record it while at the same time proposing certain simplifications and eliminating incorrect names. It hopes that the flexible system of nomenclature thus created will be used more and more by authors of articles and treatises on organic chemistry as well as in oral instruction and that the editors of journals will recommend its use as far as possible."[5]

It was largely through the interest and efforts of Drs. E. J. Crane, Austin M. Patterson, Leonard T. Capell, Mary A. Magill, and Carleton E. Curran of the Chemical Abstracts Service, a division of the American Chemical Society, that a system of naming organic compounds was worked out in this country using the rules (based on those of the Geneva Congress of 1892[5]) of the International Union of Chemistry as modified by the "Definitive Report of the Commission on the reform of Nomenclature of Organic Chemistry."

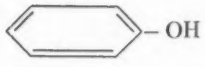
Patterson and Curran[7] explained that "we have not tried to invent a new system. Our aim has been to follow existing usage as far as it could be made fairly consistent, choosing what appeared to us good practices and rejecting bad, and introducing new features only when some very positive advantage was to be gained." The Geneva Congress of 1892 formed the basic decisions, but some of these decisions were not accepted by the majority of chemists, leaving large sections of the nomenclature without adequate guidance. The delegates to the Geneva Congress hoped to establish a single, systematic, official name for every organic compound. This goal has never been reached. Even today *Chemical Abstracts* does not attempt to assign a single name to a compound but, while following conventions in naming, strives to be consistent and unambiguous.

One important aspect of naming organic compounds is that the rules apply to all types of structures whether derived from plants and animals or synthesized. If the structure can be determined, a systematic name can be written without regard to origin or use. New names are continually being added as new com-

pounds are synthesized. A list of the most common radical names can be found in "The Naming and Indexing of Chemical Compounds by *Chemical Abstracts* (Introduction to the 1945 Subject Index including revisions in 1957)."[3] This article is a "must" for all organic-synthetic chemists interested in correctly naming their compounds. It uses the Geneva rules as modified by the International Union of Chemistry. The rule is stated and examples are given, e.g., I.U.C. rule 5: "The present names of the first four normal saturated hydrocarbons are retained. Names derived from the Greek or Latin numerals will be used for those having more than four atoms of carbon." The names used by *Chemical Abstracts* for the normal (straight chain) alkanes are:

| | | | |
|---|---|---|---|
| $CH_4$ | methane | $C_6H_{14}$ | hexane |
| $C_2H_6$ | ethane | $C_7H_{16}$ | heptane |
| $C_3H_8$ | propane | $C_8H_{18}$ | octane |
| $C_4H_{10}$ | butane | $C_9H_{20}$ | nonane |
| $C_5H_{12}$ | pentane | $C_{10}H_{22}$ | decane |

Since compounds may contain more than one functional group, e.g., $-OH$, $-NH_2$, etc., it is necessary to establish which of these groups will be used as the principle one in naming the compound. The I.U.C. rules do not indicate an order of precedence. *Chemical Abstracts* has established an order to follow. I.U.C. rule 51 reads: "For compounds of complex function, that is to say, for compounds possessing different functions, only one kind of function (the principle function) will be expressed by the ending of the name. The other functions will be designated by appropriate prefixes." Therefore, "phenol" is preferred to hydroxybenzene and aniline to aminobenzene. When the two groups are on the same benzene ring, there must be an order of precedence. Is the preferred name aminophenol or hydroxyaniline? The order of precedence is given below:

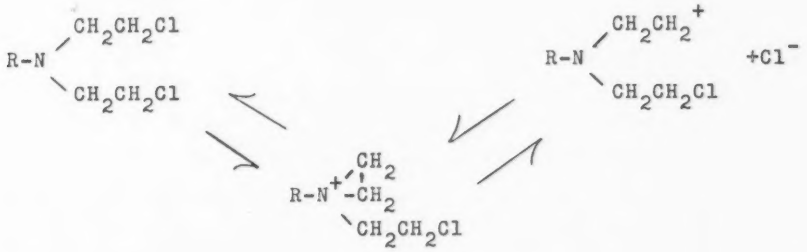
| | | | |
|---|---|---|---|
| "onium" compounds | $-\overset{\vert}{\underset{\vert}{\overset{\vert}{\underset{\vert}{N^+}}}}-$ | ketone | $-\overset{\overset{O}{\Vert}}{C}-$ |
| acid | $-COOH$, $-\overset{\overset{O}{\Vert}}{\underset{OH}{\overset{OH}{P}}}$ | alcohol | $-OH$ |
| | | phenol |  |
| acid halide | $-\overset{\overset{O}{\Vert}}{C}-Cl$ | thiol | $-SH$ |
| | | amine | $-NH_2$ |
| amide | $-\overset{\overset{O}{\Vert}}{C}-NH_2$ | oxyamine | $RONH_2$ |
| | | imine | $=NH$ |
| imide |  | ether | $-O-$ |
| | | sulfide | $-S-$ |
| amidine | $-\overset{\overset{NH}{\Vert}}{\underset{NH_2}{C}}$ | sulfoxide | $-\overset{\overset{O}{\Vert}}{S}-$ |
| aldehyde | $-\overset{\overset{O}{\Vert}}{CH}$ | | |
| nitrile | $-CN$ | sulfone | $-\overset{\overset{O}{\Vert}}{\underset{\Vert O}{S}}-$ |
| isocyanide | $-NC$ | | |

The list indicates that aminophenol is preferred to hydroxyaniline since phenol precedes the amine group. Selecting the best index compound can be difficult. Usually the largest parent is used, but there are exceptions. Diphenylmethane comes under the principle of treating like things alike. The two phenyl groups are treated alike although the parent, methane, is smaller. Otherwise the name might be α-phenyltoluene.

The inversion of the name, that is, placing the index compound name first, has the advantage of grouping closely related chemicals alphabetically. This system is used primarily for indexing when preparing an alphabetic file or a table of compounds in a manuscript, for example, 6-diazo-5-oxo-L-norleucine (DON) is indexed as norleucine, 6-diazo-5-oxo-, L-. In this way, all the norleucines will be grouped together.

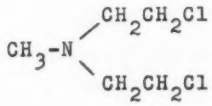
After determining the index compound, the next step is to arrange the radical groups in order. I.U.C. rules 7 and 63 permit almost any order, i.e., according to size of radical, complexity, or atomic composition, alphabetically, etc. Unfortunately, no system had been worked out for arranging the substituted radical groups. Again, *Chemical Abstracts* has set up an order for convenience and consistency. The radicals are arranged in alphabetic order, but the prefixes di, tri, tetra, etc., are not considered. Certain groups, however, are treated as units, e.g., dimethylamino, with d as the index letter, and o,p′-DDD (used for adrenal cortical cancer). In ethane, 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-, di of dichloro is disregarded in arranging these groups. The c is the alphabetizing letter in both dichloro and chlorophenyl. The position designations *o*- and *p*- are not used in alphabetizing except where there are like groups.

Many of the rules are illustrated by compounds that have been used in one or more of the neoplastic diseases. The discovery that mustard gas, bis(2-chloroethyl)sulfide, decreased the white blood cell count, which would indicate an improvement in leukemia, gave new hope to those interested in the chemotherapy of cancer.[8] Because sulfur mustards were highly toxic as well as being vesicants, researchers investigated the nitrogen analogues which were less toxic and easier to administer. The nitrogen mustards come under the category of alkylating agents, in which the reactive intermediate is said to be a carbonium ion arising from a cyclic ethylenimonium ion as follows:

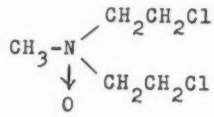


One of the most commonly used nitrogen alkylating agents of the mustard type is mechlorethamine (Mustargen), or HN2, which has been referred to as methyl bis(2-chloroethyl)amine hydrochloride. While this designation is descriptive (that is, an unambiguous name from which its structure could be written), it does not follow the rules of nomenclature that are used by *Chemical Abstracts.*

The correct name is, therefore, 2,2′-dichloro-N-methyldiethylamine hydrochloride. Its structural representation is:

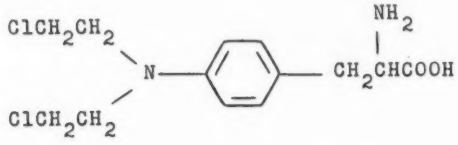


An explanation of the numbers and letter designations in the name is as follows: 2- indicates the chlorine is on the second carbon of one ethyl group, counting the carbons from the nitrogen; 2′-indicates the chlorine on the second carbon of the other ethyl group, N means that the methyl is attached to the nitrogen; the hydrochloride forms an ionic bond with the nitrogen and this changes the nitrogen from a trivalent atom to a pentavalent ammonium type ionized atom, which increases the water solubility of the compound. The N-oxide of HN2, mechlorethamine oxide (Nitromin), is represented:

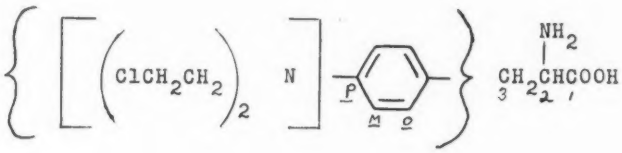


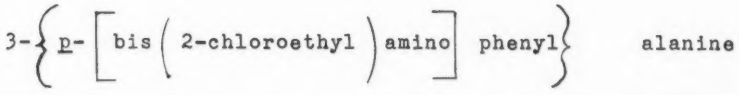
The arrow from the nitrogen to the oxygen indicates a "partial charge" on the double bond and could be written $-\underset{\delta^+}{N}=\underset{\delta^-}{O}$.

With the nitrogen mustard nucleus as a start, many related compounds were synthesized. One of these is the phenylalanine derivative:



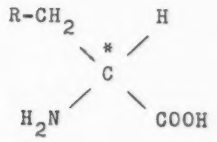
The *Chemical Abstracts* name is 3-{*p*-[bis(2-chloroethyl)amino]phenyl}alanine. For a clearer picture of what the numbers, letters, parentheses, and braces mean, they can be broken down into the chemical groups:



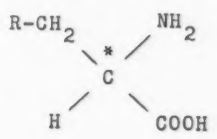



The 3 represents the third carbon of the alanine group. Everything within the braces is attached to the third carbon of the alanine parent. The *p* indicates the para or 4 position of the phenyl radical, and everything within the brackets is attached to the fourth carbon of the phenyl radical. The bis refers to the two identical groups within the parentheses. The two 2-chloroethyl groups are at-

tached to the nitrogen or amino radical. This compound can exist in two forms. The arrangement of the groups on the asymmetric carbon atom* will produce either a D or L configuration:



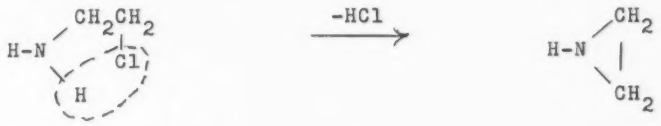
D-



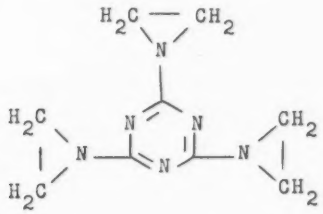
L-

A compound with two geometric configurations will have the same empirical formula but will differ in its physical and biologic properties. The L-isomer is known as L-sarcolysine or melfalan, the D-isomer is medphalan, and the DL-isomer (an equimolar mixture of D and L) is called sarcolysin or merphalan.

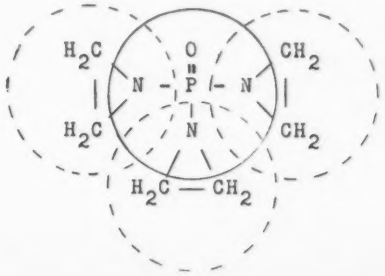
Various other structures that contain the nitrogen mustard group were explored. One important group is the ethylenimine or aziridinyl group which is formed by the dehydrochlorination of 2-chloroethylamine:



The first of these to be studied was 2,4,6-tris(1-aziridinyl)-*s*-triazine(TEM)[6]:

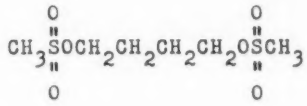


A breakdown of the name and structure is described below: The *s*- with triazine stands for symmetric, which means that there are alternate nitrogen and carbon atoms in the ring. Tris is used instead of tri before complex expressions. The 1- in the parentheses applies to the nitrogen of the aziridinyl group. The 2,4,6- refers to the substitutions on the carbon atoms in the triazine ring. The nitrogen atoms have preference over the carbon atoms in a heterocyclic system; therefore, the numbering begins with nitrogen. (A heterocyclic compound is a ring compound containing another element in addition to carbon in the ring.) Another compound of this type is TEPA:

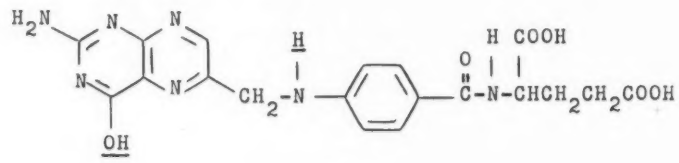


TEPA was previously called N,N′,N″-*trie*thylene thio*p*hosphor*a*mide, a name which used the atoms in the circle as the parent group (phosphoric triamide). This method of naming splits the aziridinyl rings, and rings should be treated as a unit. To keep them intact, as shown by the dotted lines, the compound is, correctly, tris(1-aziridinyl)phosphine oxide.

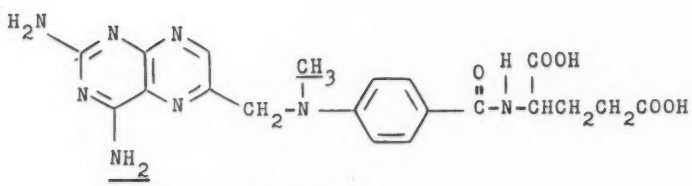
The naming of the methanesulfonate ester type is straightforward. For example, busulfan (Myleran) is the tetramethylene ester of methanesulfonic acid:



The antimetabolites are compounds with structures similar to but with physiologic actions antagonistic to essential metabolites; these have been useful in cancer. For example, amethopterin (Methotrexate) is the result of an alteration of the structure of folic acid:

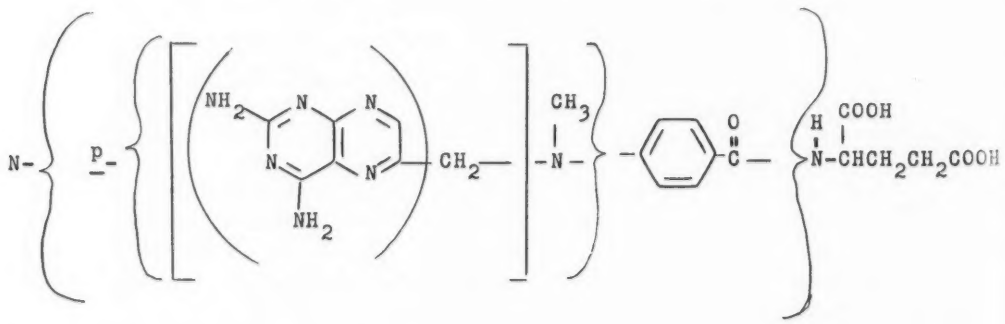


Folic Acid



Amethopterin

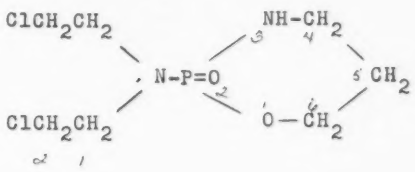
Glutamic acid, N-{*p*-{[(2,4-diamino-6-pteridyl)methyl]methylamino}benzoyl}- is the chemical name for amethopterin. By means of parentheses, brackets, and braces, the attachment of one group to another is shown:



N- { p- { [ (2,4-diamino-6-pteridyl) methyl ] methyl amino } benzoyl } glutamic acid

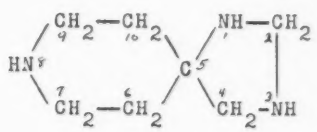
Starting with the innermost parentheses, the attachment of the methyl group to the 2,4-diamino-6-pteridyl group is shown with brackets. The attachment of the methyl group to the amino group is shown with braces. The amino group is attached to the para position of the benzoyl group, and all are enclosed in large braces, which means that all the afore-mentioned groups are attached to the nitrogen of the glutamic acid. The name of the compound is written in inverted order to emphasize the parent name, which is determined by order of preference, since acids are listed before amides, amines, or any other group in this structure. Glutamic acid, incidentally, is an accepted name for 2-*amino*-*glut*aric acid.

A cyclic compound called cyclophosphamide (Cytoxan, Endoxan) combines the nitrogen mustard group with a phosphoramide group. Chemically, it is N,N-bis(β-chloroethyl)-N′,O-propylene phosphoric acid diamide:

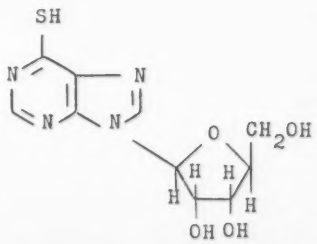


In *Chemical Abstracts*, it is designated as 2H-1,3,2-oxaazaphosphorinane, 2-[bis (2-chloroethyl)amino]-, 2-oxide. In the ring, which comprises the parent group, the 2H indicates a substitutable hydrogen on the phosphorus or 2 position of the ring. The 1 refers to oxa, or oxygen, and the 3 refers to the aza or nitrogen in the phosphorinane ring. The 2-oxide indicates an oxygen double bonded to the phosphorus. The oxa-aza nomenclature is used for both cyclic and acyclic compounds where it may provide a less cumbersome name than older methods.

I.U.C. rule 16 reads: "The ending *a* is adopted for hetero atoms occurring in a ring. Oxygen will accordingly be indicated by *oxa*, sulfur by *thia*, nitrogen by *aza*, etc. The letter *a* may be elided before a vowel. Examples: thiadiazole, oxadiazole, thiazine, oxazine. While the universally accepted names of heterocyclic compounds are retained, the names of other heterocyclic compounds are derived from that of the corresponding homocyclic compound by adding to it the names of the hetero atoms ending in *a*. Example: 1,3,8-triazaspiro[4.5] decane."



The purines are known to occur as ribosides and ribotides in nucleic acids. Variations of these antimetabolites led to the investigation of 6-mercaptopurine riboside:



The name in *Chemical Abstracts* is 9H-purine-6-thiol, 9-β-D-ribofuranosyl-. The mercapto group is treated as an alcohol and combined with the purine to form the parent name. The 9H- indicates a replaceable hydrogen in the 9 position of the purine, the point of attachment for the β-D-ribofuranosyl group.

Names in *Chemical Abstracts* will differ from many in common use, but closer examination shows that the name commonly used is often a shortened one which has lost much of its descriptive value. Although the chemist knows how to designate a compound correctly, he invariably coins a shorter name when referring to it verbally. This eventually is written into the literature, and then the confusion begins. *The Merck Index*, which lists compounds with their synonyms, contains many examples of the multiplicity of names referring to a single chemical.

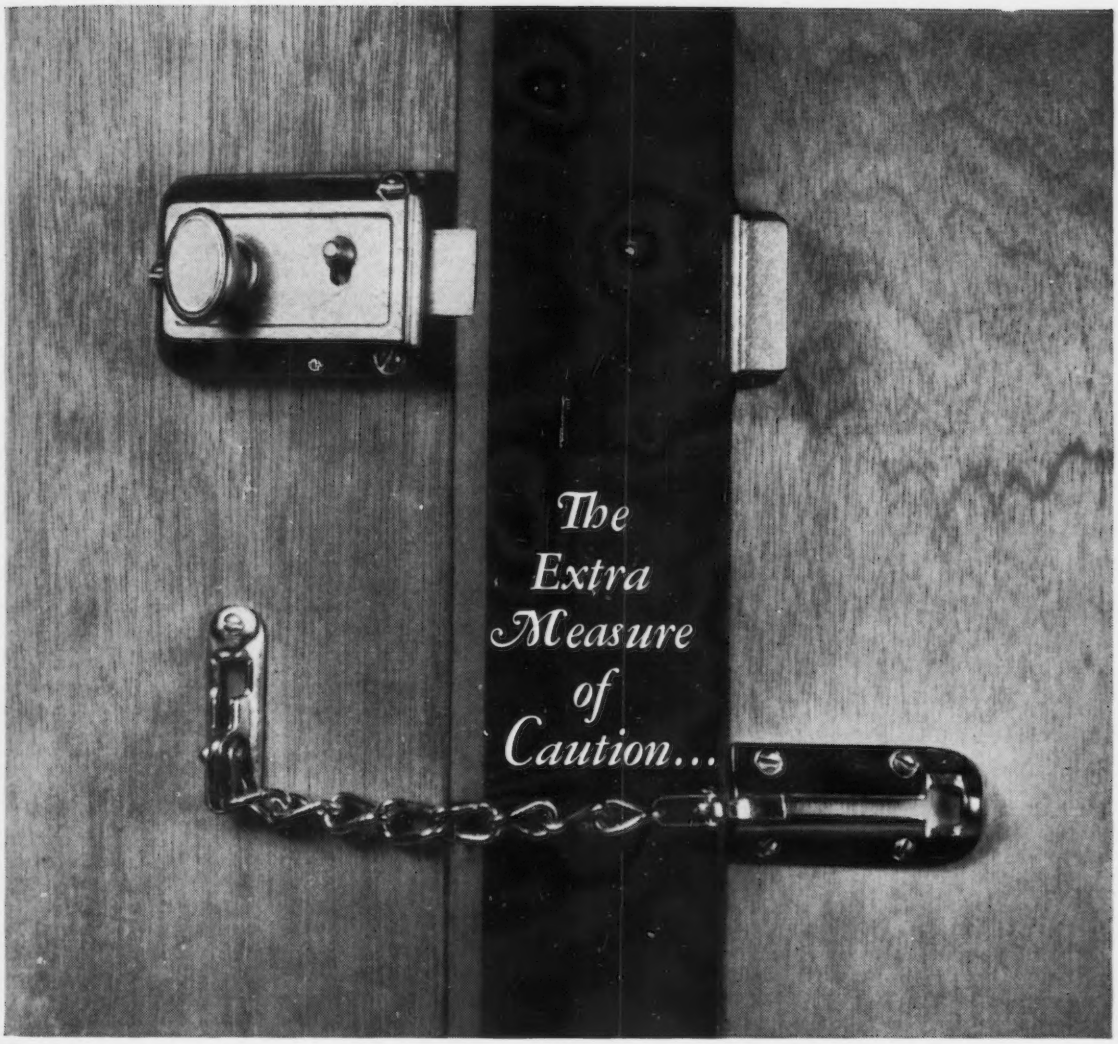
Unfortunately, not all chemical names can be as short as aniline or purine, but certain combinations of chemical groups can be named as a unit. Folic acid is an excellent example. Because of its occurrence in green leaves, the name folic was taken from the Latin word *folium*, leaf. However, substitutions on this structure usually require the compound to be named as a derivative of glutamic acid.

### *References*

1. Gamble, D. F.: Agents of interest in cancer chemotherapy research and their synonyms, Cancer Chemother. Rep. **4**:11-47, 1959.
2. The Merck index, ed. 7, Rahway, N. J., 1960, Merck & Co., Inc., p. 420.
3. The naming and indexing of chemical compounds by *Chemical Abstracts*, Introduction to the 1945 subject index including revisions in 1957, Chem. Abst. **51**:1R-28R, 1957.
4. Oesper, R. E.: The birth of the modern chemical nomenclature, J. Chem. Ed. **22**:290-292, 1945.
5. Patterson, A. M.: Definitive report of the Commission on the Reform of the Nomenclature of Organic Chemistry, Translation with comments, J. Am. Chem. Soc. **55**:3905-3925, 1933.
6. Patterson, A. M., Capell, L. T., and Walker, D. F.: The Ring index, ed. 2, Washington, 1960, American Chemical Society, p. 27.
7. Patterson, A. M., and Curran, C. E.: A system of organic nomenclature, J. Am. Chem. Soc. **39**:1623-1638, 1917.
8. Ross, R. B.: Recent advances in chemotherapy of cancer, J. Chem. Ed. **36**:368-377, 1959.

RAY W. IHNDRIS

*Cancer Chemotherapy National Service Center*
*National Institutes of Health*
*U. S. Public Health Service*




## *Tetracycline now combined with the new, more active antifungal antibiotic – Fungizone – for broad spectrum therapy / antimonilial prophylaxis*

A new advance in broad spectrum antibiotic therapy, MYSTECLIN-F provides all the well-known benefits of tetracycline and also contains the new, clinically proved antifungal antibiotic, Fungizone. This Squibb-developed antibiotic, which is unusually free of side effects on oral administration when given in oral prophylactic doses, has substantially greater in vitro activity than nystatin against strains of Candida (Monilia) albicans.

Thus, in addition to providing highly effective broad spectrum therapy, MYSTECLIN-F prevents the monilial overgrowth in the gastrointestinal tract so commonly associated with such therapy. It helps to protect the patient from troublesome, even serious, monilial complications.

New Mysteclin-F provides this added antifungal protection at little increased cost to your patients over ordinary tetracycline preparations.

*Available as:* MYSTECLIN-F CAPSULES (250 mg./50 mg.) MYSTECLIN-F HALF STRENGTH CAPSULES (125 mg./25 mg.) MYSTECLIN-F FOR SYRUP (125 mg./25 mg. per 5 cc.) MYSTECLIN-F FOR AQUEOUS DROPS (100 mg./20 mg. per cc.)

For complete information, consult package insert or write to Professional Service Department, Squibb, 745 Fifth Avenue, N. Y. 22, N. Y.

SQUIBB *Squibb Quality – the Priceless Ingredient*

NEW
# MYSTECLIN-F
Squibb Phosphate-Potentiated Tetracycline (SUMYCIN) *plus* Amphotericin B (FUNGIZONE)

'MYSTECLIN'®, 'SUMYCIN'® AND 'FUNGIZONE'® ARE SQUIBB TRADEMARKS

The basic question is whether we are to discard the system that has brought us to our present level of health care, and promises much higher levels for the future, in favor of a regulatory strait jacket that stifles initiative, bureaucratizes research, and promises nothing for the future.

# You can't go places in a strait jacket...!

An editorial writer recently made the interesting suggestion that the pharmaceutical industry might have avoided much of the current public interest in its affairs if they had simply restricted themselves to making aspirin tablets and rubbing alcohol, competing only by debating which aspirin dissolves faster. • No one has seriously suggested a return to the "good old days" in therapeutics, but there are apparently some who would like to destroy the system that has produced for us the finest medical care in the history of the world. Whether they attack the freedom of the patient to choose his physician, the freedom of the physician in the practice of his profession, or the freedom of the pharmaceutical industry is immaterial. • If the desideratum is simply maintenance of the status quo in health care, medicine might well have rested on its 19th century laurels and the pharmaceutical industry on aspirin tablets and rubbing alcohol.

*This message is brought to you on behalf of the producers of prescription drugs as a service to the medical profession. For additional information, please write Pharmaceutical Manufacturers Association, 1411 K Street, N.W., Washington 5, D. C.*

*You know all the signs but do you know...*

*How you can make best use of laboratory tests to diagnose and determine proper management*

*How you can evaluate the probable effectiveness of specific drugs*

*How you can obtain detailed directions for use of all available anti-hypertensive drugs*

*Get experienced counsel on details of therapy in*

## MANAGEMENT OF HYPERTENSIVE DISEASES

**By JOSEPH C. EDWARDS, A.B., M.D., F.A.C.P., F.A.C.C., Assistant Professor of Clinical Medicine, Cardiovascular Consultant to Division of Gerontology, and Consultant in the Hypertension and the Cardiac Clinics, Washington University School of Medicine and Barnes Hospital, St. Louis, Mo. Published October, 1960. 439 pages, 6¾" x 9¾", illustrated. Price, $15.00.**

Practical answers to the problems listed above and to many others regarding diagnosis and long term clinical management of patients with hypertensive disease are competently explained to you in this new book by an experienced and presently active clinician and educator who specializes in this area, Joseph C. Edwards, M.D.

MANAGEMENT OF HYPERTENSIVE DISEASES can help you to develop a sound program of well planned and individualized clinical management for each of your patients with hypertensive disease. It directs special attention to treatment, especially of the most commonly encountered primary idiopathic type of hypertension. The book also discusses treatment of hypertensive emergencies and prognosis of various degrees of hypertensive disease. Diagnostic methods for all forms of the disease—curable and non-curable, renal, adrenal, primary and secondary types of hypertensive diseases—are comprehensively described.

In many instances, Dr. Edwards uses actual case presentations listing clinical findings, laboratory data and kinds and doses of drugs along with the actual blood pressure responses to aid you in the management of your own patients. In a practical chapter on anti-hypertensive drugs, you'll find that all drugs—even many recently introduced ones—are evaluated and that recommended dosages, principal side reactions and possible toxic effects are explained in full detail.

*Order on 30 Day Approval. Money Back Guarantee!*

**The C. V. Mosby Company**

**3207 Washington Boulevard, St. Louis 3, Missouri**

CLINICAL PHARMACOLOGY and THERAPEUTICS

## Product index to advertisers

# CLINICAL PHARMACOLOGY and THERAPEUTICS

## articles to appear in early issues

**The hypotensive effect of imipramine hydrochloride in patients with cardiovascular disease**

*Otto F. Muller, M.D., Nelson Goodman, M.D., and Samuel Bellet, M.D., Philadelphia, Pa.*

**Factors affecting the absorption and biliary excretion of erythromycin and two of its derivatives in humans**

*James B. Hammond, M.D., and R. S. Griffith, M.D., Indianapolis, Ind.*

**Anesthetic time/dose curves. III. The interaction of thiopental and oxymorphone during surgical anesthesia**

*M. Keeri-Szanto, M.D., M. Knaff, M.D., and Y. Rondeau, M.D., Montreal, Que.*

**Clinical pharmacology of antiarrhythmic drugs**

*Samuel Bellet, M.D., Philadelphia, Pa.*

**Symposium on the experimental pharmacology and clinical use of antimetabolites**

**Part VII. The pharmacology and clinical use of the spirolactones**

*David H. P. Streeten, M.B., M.R.C.P., D.Phil., Syracuse, N. Y.*

**Part VIII. Folic acid antagonists**

*James F. Holland, M.D., Buffalo, N. Y.*